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Review Article

ASSOCIATION OF THE *SHANK3* GENE COUPLED WITH *22Q13.3* DELETION SYNDROME

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ABSTRACT

Phelan-McDermid syndrome (PMS) is a postnatal neurodegenerative disorder which is caused by the mutation in the gene called SHANK3, a member of the highly conserved Shank/ProSAP family of synaptic scaffolding proteins. The haploinsufficiency as well as genetic alterations of SHANK3 gene is responsible for the deletion of 22q13.3. The function of SHANK3 is to connect ion channels and receptors in the post-synaptic membrane to the cytoskeleton and to signal transduction pathways. So, to understand the cause of PMS, it is necessary to know the mechanism of SHANK3 pathways. Thus the article aims to explain the in depth about many aspects of clinical and genetic facets of 22q13.3 deletion syndrome, with more importance in the domain architecture of SHANK3 protein, and by shedding light on the haploinsufficiency mutations involved in the protein families related to the upstream and downstream signal transduction and its clinical effect.

Keywords: Phelan- McDermid syndrome (PMS), microdeletion, SHANK3, 22q13.3.

INTRODUCTION

Phelan-McDermid Syndrome (PMS) is a post natal neurodegenerative disorder caused by the microdeletion of SHANK3 gene on distal portion of chromosome 22q13.31. It is characterized by the developmental delay, delayed or absent speech, childhood neonatal hypotonia, autistic-like behaviour, normal to accelerated growth in absence of somatic abnormality, mild intellectual impairment, increased pain tolerance and minor dysmorphic features like dolicocephaly, thick eyebrows, lengthy eyelashes, large or prominent ears, dysplastic toenails, broad nasal bridge, bulbous nose and sacral dimple[2, 3, 4, 5] (Figure 1 and Table 1). The prevalence of PMS is largely unknown but the occurrence in male and female is the same. The genetic alterations that lead to the syndrome will mainly affect the gene SHANK3 which code for a scaffold protein localized in

the postsynaptic density (PSD) of excitatory synapses, which connects membrane receptors with cytoskeleton. Therefore, the main objective of this review is to elucidate the upstream and downstream of SHANK3 pathway in PSD and to provoke a hypothesis on how alterations in the SHANK3 protein lead to the syndrome. In addition, the frequently observed clinical, morphological and behavioural deficits of some individuals with 22q13.3 deletion have also been analyzed.

The chromosome 22 is the second smallest in human and was the first to be sequenced6.The first case of 22q13.3 deletion was reported in 1985 and it was the result of rearrangement of maternal pericentric inversion of chromosome 227. Phelan was the first person to report hypotonia in a new-born boy with a terminal de novo deletion of 22q13.3 along with changes in some phenotypic and neurological characteristics and named as Phelan-McDermid Syndrome8. About 1% of the population are affected with Autism spectrum disorders (ASD) in which the genetic factor plays a major role[9]. One of the ASD linked de novo mutation involves SHANK3 gene which affirms the genome wide association studies[10, 11].SHANK3 gene is the major contributor to 22q13.3 deletion syndrome or PMS which was reported in a child with a de novo reciprocal balanced translocation t(12;22)(q24;q13.3) that interrupted the ProSAP2/Shank3 gene in exon 2112.

The major cause for the deletion of 22q13.3 was identified as haploinsufficiency of the ProSAP2/Shank3 gene due to the loss of one ProSAP2/Shank3 copy in 22q13 region[12, 13]. Postsynaptic ProSAP/Shank scaffolding proteins are involved in maintaining synaptic homeostasis and have been linked with synaptopathies14. PMS can be detected by fluorescence in situ hybridization (FISH) using arylsulfatase A (ARSA) or D22S1726 (subtelomeric) probe, the former can detect the distal deletions and the latter detects interstitial deletions thus giving a 100% result [1, 15, 16, 17]. An earlier study with 33 individuals out of which 17 were seen with the deletion on chromosome 22 size ranging from 160 kb to 9 Mb and the critical regions included SHANK3, ACR, and RABL2B (Table 3) and also 74% of the deletions were on paternal chromosome 2218. A similar kind of study with 56 individuals obtained with the same results [13].

CLINICAL FEATURES

Patients with Phelan-McDermid syndrome appears to be neonatal hypotonic (>97%) and have global developmental delay (>98%), normal to accelerated growth (95%), absence of severely delayed speech (>98%), and minor dysmorphic features [3, 5, 12, 13, 18, 19]. The features of PMS are very rare at the time of birth. The early conferral symptoms of 22q13 deletion syndrome are hypotonia, feeding problems, and developmental delay. The patient with PMS have common physical features including long eye lashes, large or unusual ears, relatively large and fleshy hands, dysplastic toe nails, arched eyebrow, dolicocephaly, full cheeks, bulbous nose, pointed chin, sacral dimple and decreased perspiration[17] (Figure 1). The PMS patients apparently have autistic-like behaviour, poor eye contact, stereotypic movements, decreased socialization, language impairment, chewing non-food items and increased tolerance to pain [16,17,20,21,22,23]. Further, patients may also suffer from convulsions, motor deficits, deformities in brain, kidney or heart, gastro-oesophageal reflux or some immune defects[5].

Following are the earlier studies reported were a 13 year old child assessed by the Psycho-Educational Profile-Revised (PEP-R) with developmental delay[24] whereas a 14 year old female with tenuous facial dysmorphic characteristics including hypertropic nasal root, up slanting fissures, thick lips and normal oral cavity was observed[23]. Later in 2006, a 17 year old female with facial dysmorphism, brachycephaly, deep set eyes, short philtrum, mild prognatism, hypoplastic ear lobes and macrostomia was examined. Another report postulated that a patient who underwent a psychiatric examination since two years of age, showed severe mental retardation, language barriers and autistic behaviors including lack of sleep, stereotypic hand movements, loss of social interaction, lack of bowel control and end of diurnal enuresis[24]. For the first time, PMS was reported in a 70-year-old female patient diagnosed with atypical bipolar affective disorder after careful behavioral review. She was intellectually disabled with incomprehensible speech, marked thoracic kyphosis, stiff flexing in both arms and twisted hands with thumb rotated inwards. She had facial dysmorphisms like flat mid-face, big ears, thick brow and a short philtrum. Moreover she was revealed with 160 cm height and a head circumference of 56.5cm (Vineland Screener)[25, 26, 27]. In

another study SHANK3 gene has been analyzed in 128 Japanese autistic patients having symptoms like severe delayed speech development similar to 22q13.3 deletion syndrome[28]. They found a number of variants such as 6-amino acid deletion upstream of SH3 domain, a missense alteration of arginine to histidine at 656 amino acid position in the PDZ domain, and an insertion or deletion of a recurring 10-bp GC sequence located 9-bp downstream from the 3' end of exon 11.

Causes

PMS is mostly sporadic disorder (80% reported cases of de novo deletion), while 20% are due to familial with balanced translocations or other chromosomal rearrangements. People with 22ql3 deletion syndrome have either a 22q terminal deletion with a chromosome break and loss of distal segment or an interstitial deletion or deletions due to balanced translocation or other structural rearrangements.

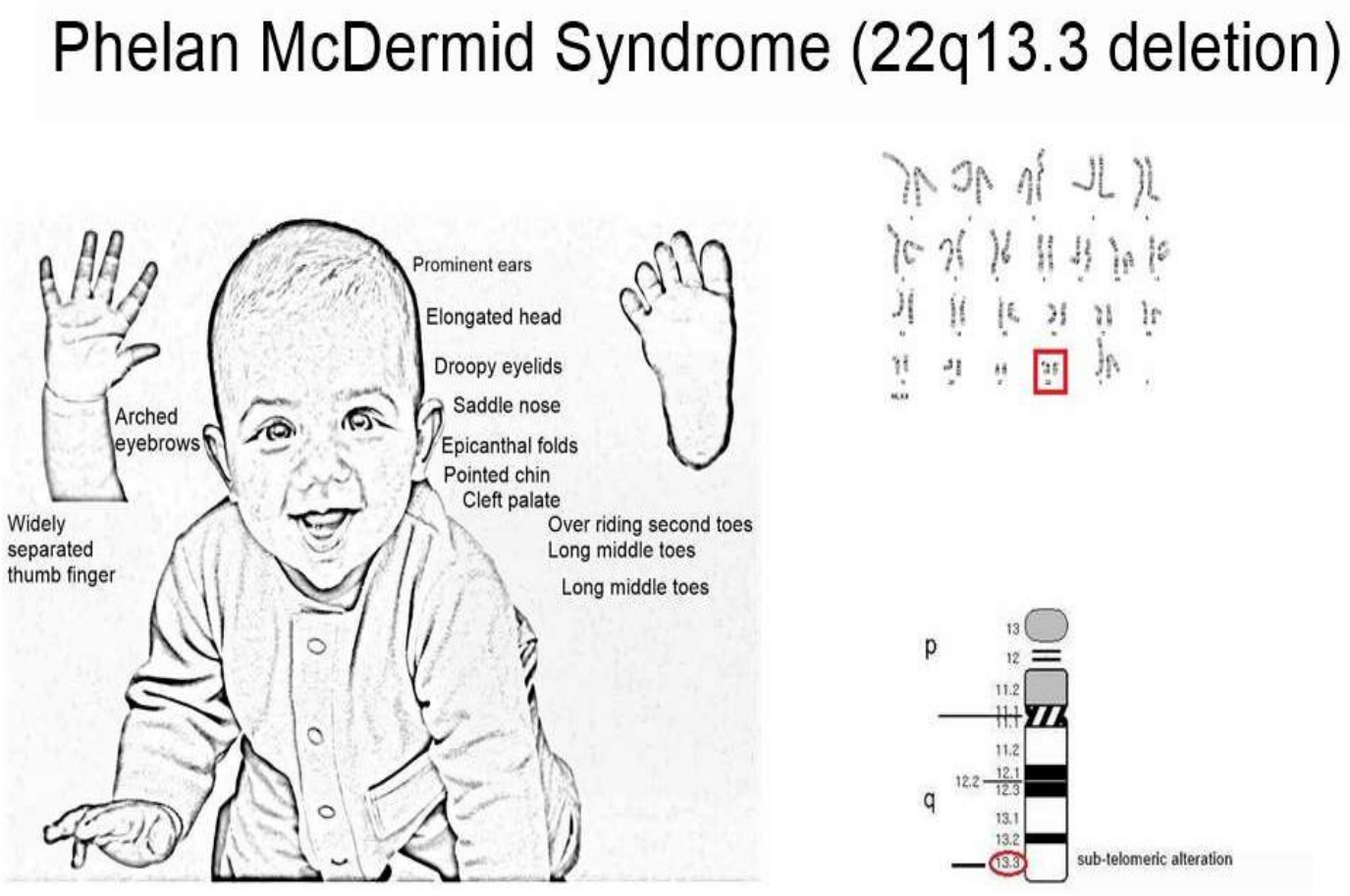

Figure1. Common physical features of Phelan-McDermid syndrome

Table 1. Comparison of the clinical and dysmorphic features

S. No	Autism	Down Syndrome	Cerebral palsy	Phelan-McDermid Syndrome
1	Developmental delay	-	-	Developmental delay
2	Mental retardation	Mental retardation, Paranoia	Mental retardation	Mental retardation
3	Language delay	Language difficulties	-	Language delay or absent of speech
4	Repetitive behaviors, Ritualistic behaviors	-	-	Autistic like behavior
5	-	Hypotonia, Dystonia	-	Hypotonia

Contd.

6	-	-	-	Normal to accelerated growth
7	Macrocephaly	Macrocephaly	-	Macrocephaly
8	Microcephaly	Microcephaly	-	-
9	Posteriorly rotated ears	Ears abnormality, Low set or dysmorphic ears	-	Dysplastic ears, Large or prominent ears, and Hypoplastic ear lobes
10	-	Epicanthal folds	-	Epicanthal folds
11	-	Hypertelorism	-	Hypertelorism
12	-	Upslanting palpebral fissures	-	Upslanting palpebral fissures
13	-	Down slanting palpebral fissures	-	Downslanting palpebral fissures
14	-	Broad nasal bridge	-	Broad and flat nasal bridge, Moderate hypertrophic nasal root
15	-	-	-	Dysplastic toenails, Syndactyly of toes with toenails
16	-	Clinodactyly	-	Clinodactyly of both 5th fingers
17	-	-	Abnormal motor behavior	Delay of gross motor milestone
18	Problems with attention and hyperactivity	-	-	Hyperactive
19	Abnormal finger digit ratios	Short stubby fingers	-	Long fingers and toes
20	Impaired adaptive and cognitive functioning	Maladaptive impairment, Changes in adaptive behavior and Cognitive decline	-	-
21	Abnormalities in sleep	-	-	-
22	-	Flat facial profile	-	Round, Long and flat face
23	Unusual mood	Depressive mood, Manic episodes	-	-
24	Compulsive behavior	Obsessive-compulsive behaviors	-	-
25	-	-	-	Shallow philtrum, Short philtrum
26	-	-	-	High arched palate
27	-	Loss of memory, Neurological changes	-	-
28	-	Excessive skin fold on neck, Short neck / increased nuchal skin thickness	-	-
29	Social interaction, Self injury	-	-	-
30	Drooling	-	-	-
31	Poor articulation	-	-	-
32	-	Renal abnormalities	-	-

33	-	Swollen feet	-	-
34	-	Talipes	-	-
35	-	Congenital heart disease	-	-
36	-	Gastrointestinal anomalies	-	-
37	-	-	-	Dolicocephaly
38	-	-	-	Brachycephaly, Large head circumference
39	-	Simian crease	-	-
40	-	Sandle sign	-	-
41	-	Protruding tongue	-	-
42	-	Hypothyroidism	-	-
43	-	-	Visual impairment	-
44	-	-	Non walking	-
45	-	-	Sensation	-
46	-	Intrauterine growth retardation	-	-
47	-	Undiagnosed cardiac murmur	-	-
48	-	Short stature	-	-
49	-	Umbilical hernia	-	-
50	-	-	-	Bulbous nose
51	-	-	-	Large hands
52	-	-	-	Arch shaped eyebrows
53	-	-	-	Prominent forehead
54	-	-	-	Saddle nose
55	-	-	-	Thick lips
56	-	-	-	Deep set eyes
57	-	-	-	Mild prognathism, Microretrognathia
58	-	-	-	Macrostomia
59	-	-	-	Low Hairline
60	-	-	-	Thin shoulders
61	-	-	-	Feeding problem
62	-	-	-	Ptosis, Periorbital puffiness
63	-	-	Hemiplegia	-
64	-	-	Quadriplegia	-

Contd.

65	-	-	Diplegia	-
66	-	-	Dyskinetic CP	-
67	-	-	Ataxic diplegia	-
68	-	-	Pure ataxia	-
69	-	-	Tetraplegia	-

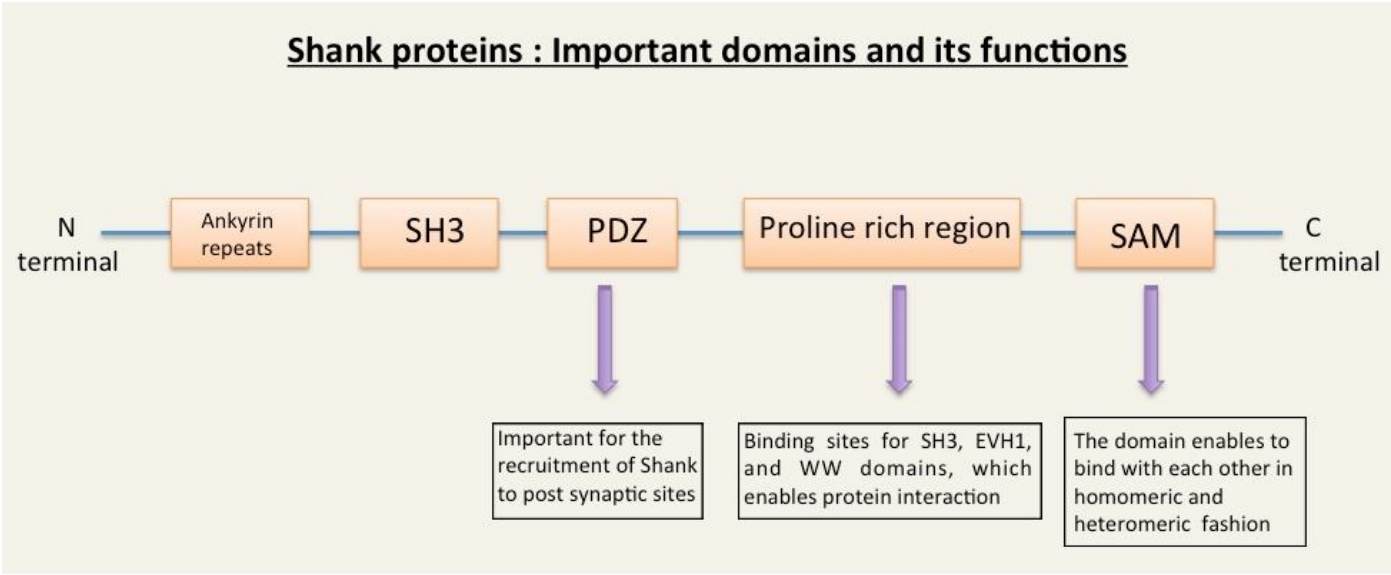

Figure 2: SHANK3 domains and their functions

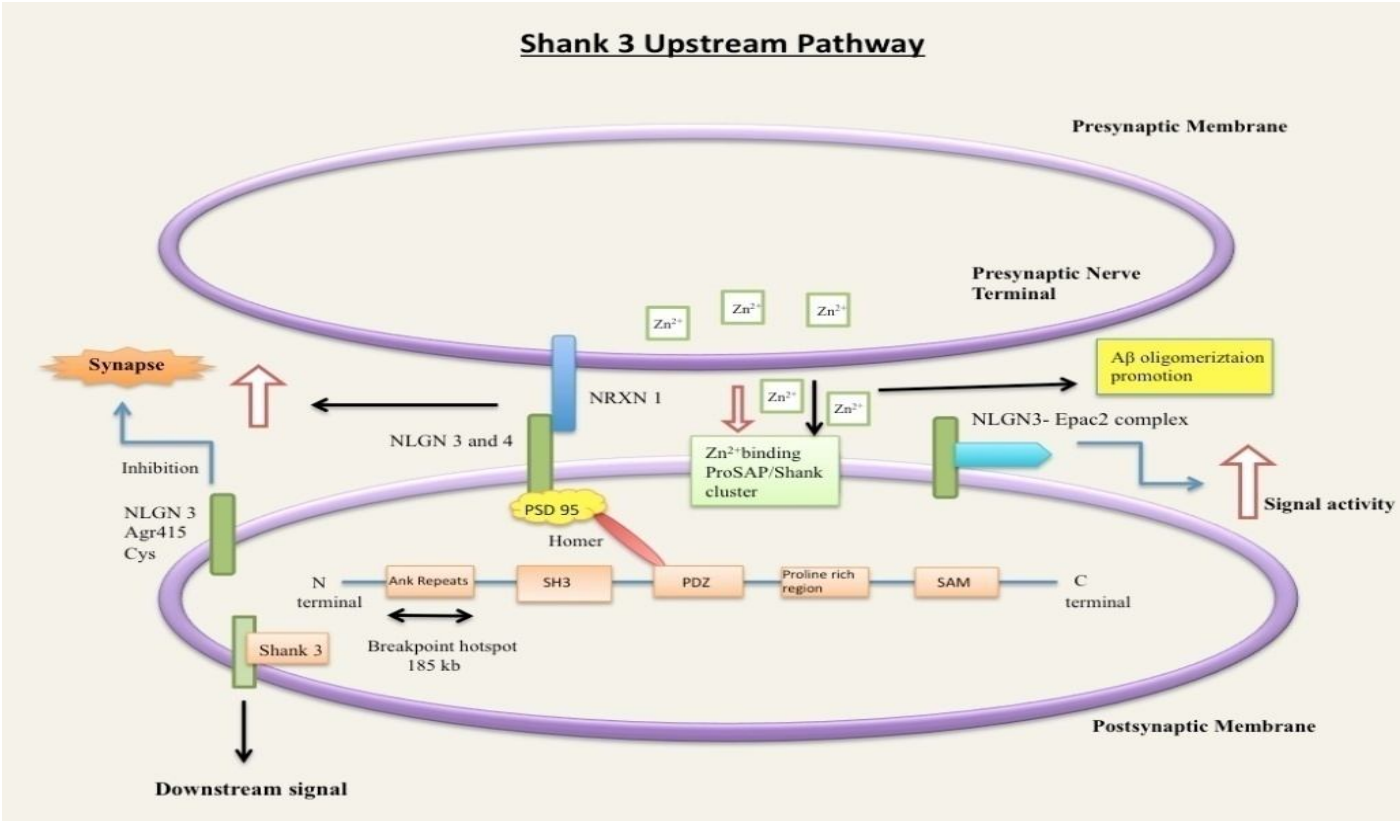

Figure 3(a): SHANK3 upstream pathway

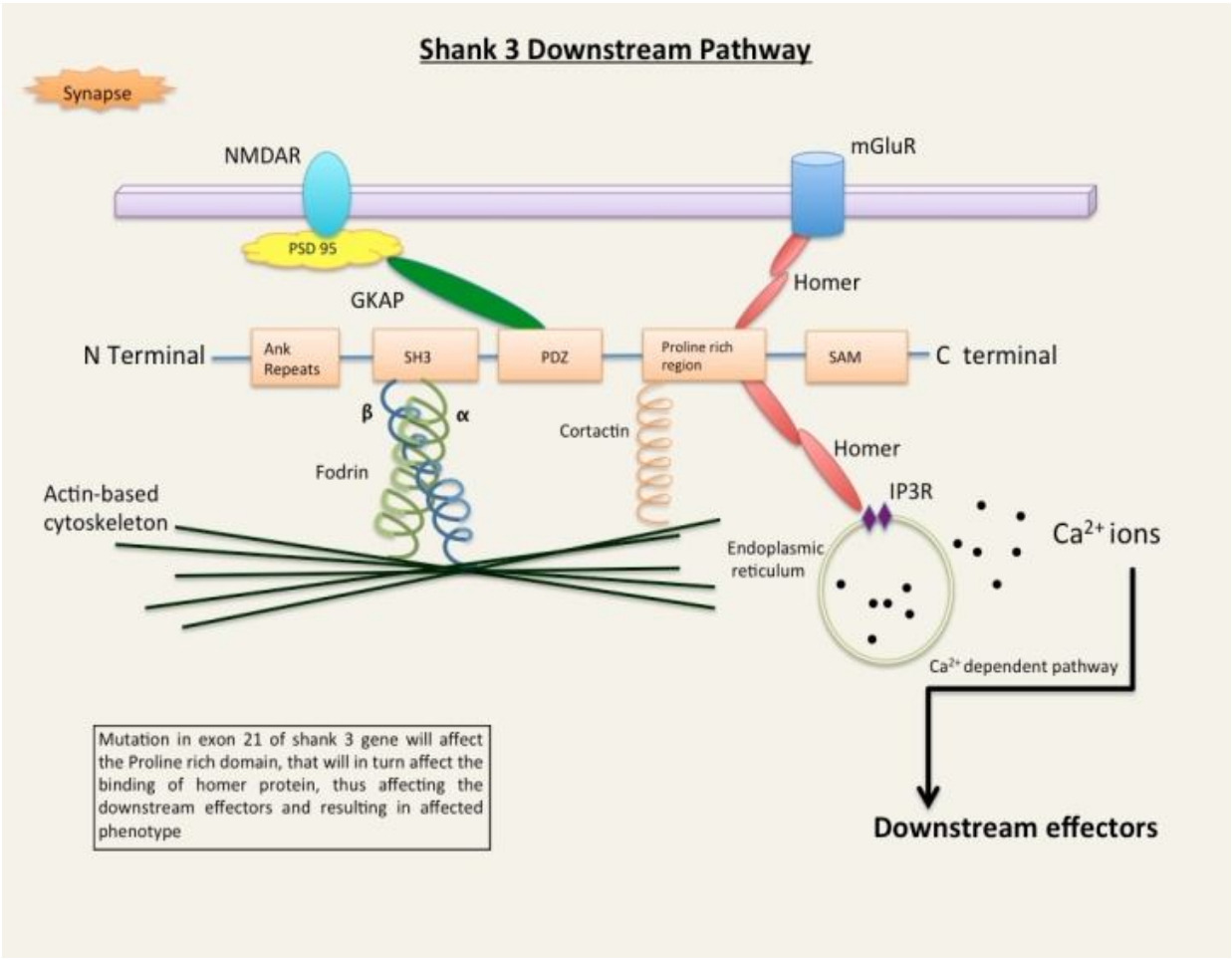

Figure 3(b): SHANK3 downstream pathway

Table 2. Genes and ASD associated proteins

S. No	Genes	Disease related	Functions	Role of genes in dendritic spine morphogenesis	Neural development	Type of Mutations	Phenotype
1	SHANK3	ASD	Synaptic scaffold	Spine number ↓	Controls spine maintenance in forebrain	Point Mutation	Developmental delay, hypotonia and absent or severely delayed speech
2	Neurexin1	ASD, Tourette's syndrome, and schizophrenia	Synaptic CAM	Spine number ↓	Mediate trans-synaptic signaling, and shape neural network properties by specifying synaptic functions	Point Mutation	Reciprocal social interaction, repetitive behavior/stereotypical patterns, obsessive-compulsive behavior and hyperactivity, macrocephaly
3	Neuroligin3	ASD, Tourette's syndrome, and schizophrenia	Synaptic adhesion	Spine number ↓	Triggers synapse formation in cultured neuronal cells	Point Mutation	Abnormality of development, mental retardation, obsessive-compulsive behavior and hyperactivity, macrocephaly
4	Neuroligin4	ASD, Tourette's	Synaptic adhesion	Spine number ↓	Triggers synapse	Promoter Mutation	Language delay, repetitive

Table 3. Functions of genes related to Phelan-McDermid syndrome

S. No	Genes	Functions	Type of Mutations
1	SHANK3	**1.** The partial loss of SHANK3 isoforms resulted with the missense and frame-shift mutations in SHANK3 ASD patients. **2.** SHANK3gene has become the focus of substantial interest and also it is the causative gene of the major neurological symptoms in the 22q13 deletion syndrome.	Missense and frame-shift mutations
2	ACR	The active enzyme causes the breakdown of the oocyte zona pellucid, so that the sperm penetration occurs.	Interstitial Deletion
3	RABL2B	The haploinsufficiency of the RABL2b in 22q13.3 deletion may play a role in syndrome phenotypes.	Interstitial Deletion
4	IB2	IB2 deficiency in mice leads to normal PSD structure but has decreased cerebellar AMPA and improved NMDA receptor associated transmission.	Terminal Deletion

SHANK3 – SH3 and multiple ankyrin repeat domains3, ACR – Acrosin, RABL2B – RAB, member of RAS oncogene family-like 2B, IB2 – Islet brain2, PSD – Post synaptic density, AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, NMDA - *N*-methyl-D-aspartate.

Haploinsufficiency of SHANK3 gene is a key reason for the neurological and phenotypic symptoms of PMS. SHANK3 (SH3 and multiple ankyrin repeat domains 3) also referred to as proline-rich synapse associated protein 2 (PROSAP2), a multidomain protein containing SH3 and PDZ domains, is thought to play an important role in the formation and function of synapses in the developing brain.

Role of SHANK mutations in PMS

Several studies had revealed the key role of postsynaptic ProSAP/Shank scaffolding proteins in molecular pathogenesis of so-called synaptopathies including autism, schizophrenia and Alzheimer's disease[14]. The ProSAP/Shank family essentially contributes to the synaptic plasticity and consists of three members: Shank1(or Shank1a, Synamon or SSTRIP), ProSAP1/Shank2 (or CortBP1) and ProSAP2/Shank3.

Shank protein has long splice variants which are ~2000 residues in length and >200 kDa in molecular mass[29]. The specific concentration of Shank proteins has been identified by Immuno-Electron Microscopy which also determined SHANK3 as a component of PSD-95/GKAP/Homer1 complex in the postsynaptic densities of excitatory synapses[29,30,31]. Shank genes showed a varied pattern of expression. SHANK1 mRNA and protein is mainly expressed in brain whereas lower level is seen in kidney and liver whereas SHANK2 mRNA is strongly seen in brain, liver, placenta, kidney, lung, pancreas and lymphoblastoid cell lines except heart and skeletal muscle[32,33,34,35].SHANK3 is highly expressed in the heart and slightly in brain[32]. Shank is a 'master scaffold' which interacts with many scaffolding proteins like GKAP, Homer/Vesl, Cortactin and GRIP thus holding together the NMDA-, mGluR- and AMPA receptor complexes in the postsynaptic specialization[29,30,36,37,38]. It has a major role in intracellular assembly of postsynaptic densities (PSDs) where the PSD lies very close to the cytoplasmic membrane[28].

Shank proteins contain five domains for protein-protein interactions and they are highly enriched in PSD. This includes an ankyrin repeat domain, a Src homology 3 (SH3) domain, a PSD-95/discs large/zonula occludens-1 (PDZ) domain, several proline-rich regions and a C-terminal sterile α-motif (SAM) domain29 (Figure 2).Shank proteins with proline-rich region bind with mGluR-binding protein, homer actin-binding protein, Abp-1 and cortical actin-binding protein which aids in polymerization of the actin cytoskeleton, an important modulator of long-term synaptic plasticity[30, 37,39,40,41].

It was discovered that SHANK3 is the host gene of neurological disorders and it can be identified as a good

candidate for finding the molecular mechanism of AMPA receptor trafficking during synaptogenesis by using yeast two-hybrid method which resulted in deduced mShank3 protein which showed 98% sequence identity with rat Shank3 [28, 29, 32, 42, 43]. Previous report suggests that there was a partial loss of SHANK3 isoforms from missense and frame-shift mutations in ASD patients but it is still unclear that whether SHANK3 point mutations behave as a loss or gain of function in them. Thus a major role was played by SHANK3 in PMS and ASD[21, 44, 45, 46].

Vigilant analysis of the extent of deletion in independent cases indicated a small critical region encompassing SHANK3. An analysis of 33 patients with different types of monosomy of chromosome 22 showed 12 cases with simple deletions of variable size (160 kb to 9 Mb), with a minimal critical region on SHANK3, ACR and RABL2B[18]. Likewise, an analysis of 56 patients with the deletion syndrome established a variable size of 130 kb to 9 Mbon SHANK3 and the minimal deleted region was seen with the same above mentioned genes.

A translocation with a breakpoint in SHANK3 causing the disruption of SHANK3 is one of the causes associated with 22ql3 deletion syndrome[12]. Similarly two more studies demonstrated a breakpoint within the same 15bp repeat unit[2] and a breakpoint in SHANK3 which overlapped with the repeating unit[47]. It was concluded from the findings that the disruption of the single SHANK3 gene is enough for the development of 22ql3 deletion syndrome.

Direct mutations in SHANK3 also result in 22q13 deletion syndrome. A de novo splice site variant of the SHANK3 gene was reported in a patient with mental retardation and severe speech impediment[48]. In a study, 427 ASD patients had a de novo deletion in SHANK3 at an intronic donor splice site and a missense variant transmitted from an epileptic father was identified[49]. Thus, various studies substantiated and implicated the single gene SHANK3 in 22ql3 deletion syndrome. It was indicated that haploinsufficiency of the gene due to the chromosomal abnormality or a mutation is primarily responsible for the phenotypic changes.

SHANK3 upstream protein pathways

The changes in the functional levels of a number of synaptic proteins cause defects in cognition and behavior in human disease. SHANK3 interacting proteins comprise of receptors, cytoskeletal proteins, scaffolding proteins, enzymes, ion channels, and signalling molecules[50,51]. It was observed that various multimers are formed using C-terminal SAM domain and PDZ domain of Shank3[29,52,53,54]. The SAM domain contains a Zn2+ bindingsite essential for SHANK3 protein folding at PSD and also for synaptogenesis and in vitro synapse maturation[50,55].

The protein expression levels can be different in individuals due to the copy number variations (CNVs)[56]. SHANK3 gene duplication of 22q13 region is the best example demonstrating that 50% increase in the level of dosage-sensitive synaptic proteins is linked to the cognitive diseases as well as 50% decrease from the loss of mutations resulting in severe impairment of social communication[20]. Stimulation of mTOR affects the initiation of eIF4E binding proteins by phosphorylation which also results in the activation of MEK/ERK. In some cases specificity is intermediated by the mechanisms concerning specific mRNAs for the translation apparatus, it affects through some general factors such as eIF4E, 4EBPs, eIF2a and eEF2 that might be evaluated to the global change in local translation[57,58,59,60,61].

Translational regulation in neurons has revealed that it is due to a spatial control mechanism which is dependent on new protein synthesis rather than cell body to change the potential of local groups of synapses in response to local input. Thus, during localization, specific mRNAs should be transferred to neuronal processes to control protein over-expression. Factors which regulate translation can be divided into three classes: (a) general translation factors, like initiation and elongation factors controlled by phosphorylation, (b) sequence-specific RNA binding proteins (RNABPs) and (c) small non-coding RNAs such as miRNAs regulating translation of specific sets of mRNAs[62, 63,64,65,66].

Two studies have introduced a mechanism and arrived at different conclusions, whether FMRP (fragile X mental retardation protein) inhibits elongation or initiation of mRNA[67,68].Thus, a study showed that FMRP interacts with the length of the coding region of the target mRNAs and arrest ribosome translocation during suppression of the translation [69]. The latest studies on the role of Aplysia FMRP homology (ApFMRP) in sensory to motor neuron synaptic plasticity which was supported by both presynaptic and postsynaptic role of

FMRP in regulating protein synthesis in response to synaptic stimulation[70, 71].

For mRNA ligands in-vitro sites were selected using either microarray or PCR[71, 72, 73]. This kind of selection explained that FMRP binds a G-quadruplex RNA motif via its RGG box and a kissing complex RNA motif occurs through the disease-associated KH2 domain[74, 75, 76]. Recent studies of FMRP on neuronal polyribosomes introduces HITS-CLIP which confirms that approximately 50% of the mRNAs identified by RIP-Chip are directly bounded by the FMRP and have extended this set of targets to more than 800 for FMRP which is directly bounded in in-vivo[69].

A covalent bond can be introduced between RNABP and RNA using UV-crosslinking areas where RNABP-RNA complexes in intact cells or tissue with stringent immunoprecipitation (CLIP) is carried out for the purification of RNABPs away from non-specific cellular RNABPs [77, 78, 79]. Synaptic cell adhesion proteins neuroligin 3 and 4 (NLGN3, NLGN4) and their presynaptic ligand neurexin1 (NRXN1) are genetically associated with autism[80]. Particularly, NLGN3 complexes with Epac2 and increases its signalling activity and SHANK3 may become a complex with NLGNs having a downstream signal [81, 82] (Figure 3(a)). In ASD synaptic pathology the emerge of different molecular and genetic subjects comprises of small GTPase and adhesion-related signalling pathways [83, 84].

Different array of frameshift, truncation and missense mutations comprises of SHANK3 mutations in autism and moreover the de novo CNVs and inherited point mutation have been identified in ASD and mentally retarded patients having SHANK2 [20, 85]. Spine sustenance in forebrain is controlled by SHANK3 whereas SHANK2 is associated in activity-dependent spine remodelling [86, 87]. Finally in the autistic individuals the rare structural variants of RAPGEF4, encoding synaptically localized Rap guanine nucleotide exchange factor (GEF) Epac2 is identified[88].

The maternal duplications of chromosome 15q11–q13 of the gene UBAE3A is the invading region of Angelman syndrome, which is related to autism[89]. The potential link between Angelman syndrome, autism and altered synaptic structure is the decrease in the spine density and length in cerebellar and hippocampal pyramidal neurons due to the deficiency of UBE3A encoding E3 ubiquitin ligase[90]. Neuregulin binding the postsynaptic receptor tyrosine kinases are known as ErbB receptors which functions in improving spine structure; pyramidal neuronal spine density and the preponderance of spines with mature phenotypes were increased by the continual NRG1 treatment[91, 92]. Thus, the knockdown of ErbB4 gene slows down the spine density and size in a cell-autonomous fashion[93].

The polymorphisms and frameshift mutations of DISC1 are linked to schizophrenia (SCZ) [94]. DISC1 gene in cortical neurons is known to interact with the regulators of spine morphogenesis, RacGEF kalirin-7 activates the downstream effectors of Rac and directly regulates the effect of DISC1 in spine morphology[95].Cellular studies exhibit a clear proof that soluble Aβ oligomers disrupt synaptic signalling which includes spine dysgenesis, and reduce spine density[96, 97, 98]. Spine loss in layer 3 prefrontal cortex neurons is explained by the decrease of kalirin[99]. In the spine the genes like ErbB4 and DISC1 interact with PSD-95 [95, 100].

In the brain of individuals with Alzheimer's disease and in transgenic animal models, actin is stabilized in spine by a postsynaptic protein drebrin[101]. Downstream effector molecule of Rac is p21-activated kinase (PAK) which is used to determine the pharmacological inhibitions of actin assembly in spine[102, 103]. In Alzheimer's disease-related pathology, depression of synaptic activity is caused by the activation of calcineurin (CaN or PP2B) which is a calcium-sensitive phosphatase associated with synaptic plasticity and also in increasing the activation of GSK-3β, a downstream effector molecule of calcineurin[104, 105]. By excluding calcineurin, Aβ oligomer–induced spine loss and dendritic dystrophies can be prevented[104, 106]. By an imbalance of synaptic plasticity mechanisms, Aβ oligomers develop the synaptic degeneration by acting on spines[96, 107].

SHANK3 downstream protein pathway

Tuberous sclerosis complex (TSC) disorder caused by mutations in hamartin (TSC1) or tuberin (TSC2), with central nervous system involvement is attributed by a high incidence of ASDs (25%-60%), cognitive impairment and epilepsy [108, 109]. In the mitotic cells (mTORC1), a major regulator of cellular growth is activated by a sequential kinase cascade downstream of phosphoinositide-3 (PI3) kinase [110]. Thus, the activation of cap-dependent translation is the major effector mechanism of mTORC1 and activation of cap-dependent

initiation is resulted by the phosphorylation of 4E-BPs by mTORC1 that alleviates this inhibition, and progressing eIF4E release. mTORC1 activity is up-regulated by the inactivation of TSC1/2 in hippocampal neurons directing to the loss of TSC1/2 function that evolves enhanced translation in the neurons [111, 112, 113]. The activity of mTORC1 is increased by the loss of PTEN function in neurons, converting the second messenger PIP3 to PIP2 which antagonizes PI3K-dependent signalling [114].

Gq-coupled metabotropic glutamate receptors mGluR1 and mGluR5 and NMDARs are the two types of postsynaptic glutamate receptors identified in translational regulation. The explorations of BDNF (which activates NMDAR)-induced LTP (Hippocampal long-term potentiation) and mGluR-dependent LTD (long-term depression), both of which requires protein synthesis, supported by isolated dendrites further advised as an important role for translationalregulation in response to synaptic activity (Figure 3(b)) [115, 116]. The suppression of mTORC1 activity in hippocampal neurons dissipates the synaptic activity-induced translation, 4E-BP phosphorylation, L-LTP and BDNF-induced LTP [117, 118]. A second independent synaptic pathway is introduced by conversion of E-LTP to L-LTP by the introduction of L-LTP in one synaptic pathway within the same neuron where proteins are synthesized in response to stimulation of one group of synapses and are available to other stimulated synapses and vice versa [119]. Inactivation of mutations in neurofibromin (NF1) causes Neurofibromatosis type I, a Ras GAP, which causes regulation of Ras-dependent ERK and mTOR activation [120].

The neuroligin (NLGN) and the glutamate receptors bind to scaffolding proteins of the post-synaptic density (PSD) such as SHANK3 on the post-synaptic side for organization of dendritic spine[121, 122]. Morphology of the neuron and the actin dynamics are regulated by TSC1 and NF1. The chromatin structure is modified by the MECP2 regulating gene expression[123].

Modelling SHANK3 and related disorders using mice

SHANK3 gene has become the limelight of substantial interest and also the major reason for many of the neurological symptoms in the 22q13 deletion syndrome [2, 13, 20, 44, 45]. Thus, the mutant mice dealing with thein vivo role of the SHANK3 in striatal glutamateric synaptic structure, function and also links the disruption of the gene as well as the autistic like behaviours in mice.

SHANK3 knockout mice shows abnormal social behaviours, communication patterns, repetitive behaviours, and impairments in learning and memory [46, 123, 124]. SHANK3 as SHANK3 duplication in mice leads to hyperactivity and spontaneous seizures much like human subjects which have small duplications in the SHANK3 locus. These recent studies further underscore the function of SHANK3 in neuronal function and possibly in the maintenance of a balance between the excitatory and inhibitory (E/I) synaptic mechanisms [125].

Expression of SHANK3 with deletions of various domains in cultured mouse neurons showed that each domain had a specific function in dendritic spine development [86]. For instance, SHANK3PDZ domain mutation led to a diminution in dendritic spine formation whilst ANK-SH3 domain alteration leads to the growth of spines with normal length but reduced head area. On the contrary, cortactin-binding site mutation produced longer spines with reduced spine head area.

In another study, knock-down of SHANK3 in cultured rat neurons selectively reduced synaptic mGluR5 receptor and caused damage to the mGluR5-dependent signalling and plasticity[126]. Excess mGluR5 signalling is linked to fragile X syndrome, which has been clinically related with 22q13.3 deletion[4, 127]. It was observed that SHANK3 mutation leads to loss of spines, decline in spine volume and reduced PSD thickness in adult. These effects, along with spine elongation, imply a phenotype of reduced or delayed synapse maturation that is indicative of phenotypes observed in mouse models of fragile X syndrome, Rett syndrome and Angelman syndrome[128].

In a study, two mutant alleles for SHANK3 gene were generated. Both mice displayed different levels of severity in the synaptic defects and phenotypes.At the ankyrin repeats and downstream of PDZ domain, multiple mutations/variants of SHANK3 gene were found in humans[21, 45]. Generally SHANK3 mutations in PMS and ASD are heterozygous in clinical conditions but in this study a homozygous mutant mice was used to understand the physiological role as well as the underlying functional consequences of disruption in the gene.

The deletion of the Homer-binding domain at the C-terminus

(ΔC) is the cause of mutations in ASD patients and thus, a model mouse is generated to determine the interaction between Shank3-Homer[20]. Recently, a mouse model was created without C-terminus of ProSAP2/Shank3. These mice displayed behavioural patterns associated with autism and SCZ. SHANK3 protein lacks the entire C-terminal portion of exon 21 which creates a mutation at the Homer protein binding site in the proline-rich domain and the SAM domain [129]. It was observed that the combination of five intragenic promoters and alternatively spliced exons resulted in a complex pattern of mRNA species both in mice and humans, and a sizable number of Shank3 protein isoforms with particular protein domain structures were yielded, including some of which are neuronsor astrocyte specific[130]. The ANK domain is physically removed by the deletion of exons 4 to 9 but it also dissolved other domains encoded by the SHANK3a and SHANK3b isoforms by the frame-shift change.

Neurotransmittance of SHANK3

SHANK3 proteins interact with neuroligins which binds to synaptic membranes and assist neurotransmission. Decrease in glutamatergic synaptic transmission and plasticity, shortfall in AMPA receptor mediated transmission and spine repair was observed in mice with SHANK3 disruption. Reduced number of GluR1-immunoreactive puncta in the striatum radiatum was also determined. These mice were lacking in social interaction and social communication. These results assert the function of SHANK3 in synaptic function, which leads to behavioural changes akin the SHANK3 mutation individuals. The 22q13 syndrome is a result of overlap of rearrangements of minimum 100 kb region between cosmid n66c4 proximally and cosmid n94h12 distally. The clone n66c4 is distal to the ARSA locus and overlaps the 5' half of SHANK3, while clones n85a3 and n94h12 and overlap the 3' end of SHANK3 and ACR.

The synaptic development and function in SHANK3 heterozygotes reveal through electrophysiological studies of the mice that decrease in basal synaptic transmission when assessed by comparing either the I/O relationship using field recordings or the amplitude of miniature excitatory postsynaptic currents (mEPSCs) using patch clamp recordings. Reduction in the AMPA receptor-dependent transmission is likely interposed by a decrease in the number of synaptic AMPA receptors. Decrease in the density of GluR1-immunoreactive puncta, persistent with a reduction in AMPA receptor levels were observed in SHANK3 heterozygous mice. The increase in presynaptic release was consistent in SHANK3 heterozygous mice with both increase in mEPSC frequency and decrease in paired pulse ratio. According to a recent report, the development of the SHANK3-deficient model has demonstrated that shorter products are truncated at the N-terminus in human and mouse SHANK3 gene which has additional start sites[130]. In PSD fractions and in mouse brain extracts, there was a strong expression of single form and slight evidence for high expression of shorter forms [131].

Diagnostic methods

Standard karyotyping or FISH is essential to detect unbalanced rearrangements in parents or other family members. Most of the deletions of 22q13 are detected using high resolution chromosomal analysis at or above 550 band level along with other accurate methods such as FISH. The arylsulfatase A (ARSA) probe successfully detects majority of 22q13.3 deletions.Microdeletions distal to ARSA were confirmed using 22q sub-telomere probe [4, 18, 20]. Identification of a de novo 22q13.3 deletion was carried out by FISH in a patient displaying developmental delay with autistic syndrome including social and communicative impairments and very presumptuous signs of deviant development with mental retardation, subtle dimorphic traits, kidney malformation, lack of flexibility, stereotyped movements with no adapted responses to emotional situations [23, 132]. Differential diagnosis includes syndromes associated with hypotonia, developmental delay, speech delay and/or autistic-like effect (Prader-Willi, Angelman, Williams, Smith-Magenis, Fragile X, Sotos, FG, trichorhinophalangeal and velocardiofacial syndromes, autism spectrum disorders, cerebral palsy).

As the whole genome can be scanned by the array-based comparative genomic hybridization (CGH) for the loss of the genetic material from 22q13 and the gain of material from a second chromosomal region, it is more acute than FISH [2, 4, 21]. Array-based comparative genomic hybridization is used to identify deletions and unbalanced translocations when the genomic arrangements are having resolution 5-10 times higher than a routine chromosomal analysis.

Genes other than SHANK3 in PMS

The 22q13.3 distal deletion also include genes other than SHANK3 such as ACR (acrosin) and RABL2B (RAB, member of RAS oncogene family-like 2B) present before the telomeric sequences which are the last two genes and IB2 (islet brain 2) also known as MAPK8IP2 (mitogen-activated protein kinase 8-inter-acting protein 2) which is present at ~70 kb nearest to SHANK3 (Table 3). Sperm acrosome contains an enzyme serine protease which is coded by ACR gene [133].The active enzyme causes the breakdown of the oocyte zona pellucida, so as to enable sperm penetration. Therefore, this gene contributes to this syndrome. RABL2B comes under the super family of RAS GTPase which is close identical paralogue to 2q13 [134]. The RABL2B gene on chromosome 22 is expressed preferentially in all the tissues tested particularly in brain and placenta [135]. IB2 gene is expressed in brain, and the protein is enriched in PSDs of mice [136]. IB2 deficiency in mice leads to normal PSD structure but has decreased cerebella AMPA and improved NMDA receptor associated transmission. Some of the autistic features of mice with other mutated PSD genes are motor deficits, decreased social interaction and exploratory behaviour[137].

SHANK3 mutations in other neuropsychiatric disorders

ProSAP2/Shank3 was the first ProSAP/Shank family member directly related to ASD/ID-associated genomic mutations showing deletions or duplications of larger areas as well as point mutations. ProSAP2/Shank3 mutations have also been linked to SCZ. In a study screening a group of SCZ patients, two unrelated individuals heterozygous for mutations of the ProSAP2/Shank3 gene, C3349T and C1606T were identified. Like ASD-associated InsG3680, C3349T, a nonsense mutation, results in shortened Pro-SAP2/Shank3 protein lacking part of its C-terminus, possibly disrupting synaptic localization and spine induction.

Introduction of different molecular techniques has led to define new genetic syndromes resulting from subtelomeric region that is distal microdeletions. Examples of some best known distal deletion syndromes are 22q13.3 [47]; 1p36 [138]; 2q37.3 [139] and 3q29 [140]. 22q deletion has been unexpectedly observed in some patients referred for DiGeorge/velo-cardio-facial syndrome (DiGeorge/VCFS)1. Some of the deletions from paternal region cause Wolf-Hirschhorn syndrome (4p deletion) [141]; cri-du-chat syndrome (5p deletion) [142]; 9p deletion syndrome [143] and 18q deletion syndrome [144]. Schizophrenia results from new mutations. Thus, two de novo mutations determined in the gene encoding SHANK3 synaptic scaffolding protein in patients confirmed SCZ. Many scientific studies have been proposed which demonstrates that SHANK3 is involved in the phenotypes of SCZ. In 2007 a girl was diagnosed with borderline intellectual functioning 22q13.3qter duplication including the SHANK3 gene and was reported as the discomposed subtype of SCZ without any familial neuropsychiatric disease [145]. In Alzheimer's disease, Shank protein which presents glutamate receptors at excitatory synapses are altered [146] (Table2).

Epigenetic regulation of SHANK3 expression

Various epigenetic mechanisms like genomic imprinting, DNA methylation, epimutations and histone modifications are linked with the development of several human neurodevelopmental disorders and normal brain development [147]. One of the mechanisms to regulate gene expression is the tissue-specific methylation. Five CpG-islands have been recognized in SHANK3 gene and tissue-specific expression of SHANK3 is regulated by DNA methylation in an epigenetic manner. In CpG islands about 60% of human genes and upto 40% of tissue-specific genes were assisted [148]. DNA methylation is carried out by the transfer of a methyl group to cytosine in a CpG dinucleotide by DNA methyltransferases [149, 150]. SHANK3 expression is influenced by the changes in CpG methylation but it is not present in other members of the human SHANK gene family while tissue-specific methylation is conserved in mouse and rat [151]. Previous reports showed the possibility to modulate the level of DNA methylation in cultured cells by increasing methionine concentration in the medium or by treating the cells with de-methylating agents such as 5-AdC [56, 152]. In hippocampal and HeLa cell cultures, Western blotting was carried out to test the expression of SHANK3 after increasing or reducing DNA methylation [31, 42]. Thus, the expressed SHANK3 in cultured neurons got decreased when induced by the high-dose methionine treatment with a subsequent decrease in dendritic spines number which determines that at least in cultured hippocampal primary neurons DNA methylation regulates SHANK3 expression [43].

Genetic counselling

About 80% of individuals with PMS have de novo mutations and simple deletions of 22q13 which can be easily detected in the earlier stages of pregnancy by parental chromosomal analysis. As in most of the terminal deletion syndrome, in PMS the deletion preferably occurs on paternally derived chromosome 22 [13, 17, 18]. Structural abnormalities were commonly related to 22q13 deletion syndrome with unbalanced translocations and ring chromosomes. The balanced translocation involving chromosome 22 in either father or mother significantly increase the risk of recurrence in future pregnancies. Several families have experienced multiple cases of 22q13 deletion secondary to familial chromosome translocations. In a case of mother to son transmission, an insertional translocation resulted in 22q13 deletion in son [152]. Thus, prenatal testing for high risk pregnancies should be done.

CONCLUSION

Phelan-McDermid exhibits vast number of clear cut indications of clinical and behavioural features. Abalanced translocation in chromosome 22 in either parent significantly increases the risk of recurrence in future pregnancies. Deletions are caused mainly by the haploinsufficiency of the SHANK3 gene which results in the decrease of synaptic transmission and reduced social behaviours. SHANK proteins show differential, although partially overlapping, expression patterns observed in the postnatal brain development. Several studiesdemonstrate the effect of SHANK3 on dendritic spine synapses by its knock-down in hippocampal neurons, which emerged for the better understanding of the genetic architecture of PMS and its molecular mechanism. Thus, advances in research may find a strong potential clinical solution. Hence, there is a need to develop strategies for advancing diagnosis, prognosis, and counselling for patients and families. Therefore, the present review provides some evidences that SHANK3 mutations and its haploinsufficiency have been clearly linked with PMS. As PMS is the most uncommon syndrome, its etiological diagnosis will give the at most benefits to the affected individuals and their families. The current review elucidates the upstream and downstream pathways of SHANK3 and its signalling alterations leading to 22q13.3 deletion syndrome based on its clinical and behavioural deficits. In forthcoming years further studies are needed in order to understand the inheritance, therapeutic targets, protein dysfunctions, chromosomal alterations and gene mutations in PMS and related disorders.

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