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The genetic architecture of monogenic neurodevelopmental disorders in Pakistan: a systematic review, 2006–2024

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Abstract

Neurodevelopmental disorders (NDDs) are among the leading causes of lifelong disability worldwide. The risk of autosomal recessive NDDs is increased in endogamous populations like Pakistan. However, limited population-specific genetic data continue to hinder accurate molecular diagnosis in Pakistan.

A retrospective systematic review was conducted to identify research articles reporting genetic findings in Pakistani families affected by NDDs between 2006 and 2024. Articles retrieved from PubMed, Google Scholar, and Web of Science were screened according to predefined eligibility criteria. Extracted data included demographic and clinical characteristics, implicated genes, and detailed variant annotations. Reported variants were cross-checked against ClinVar and evaluated using ACMG/AMP-based classification criteria. A total of 145 studies involving 537 families were included. Consanguinity was reported in 85% of the pedigrees, and whole-exome sequencing was the predominant molecular approach, accounting for approximately 85% of the studies. Overall, 424 unique genetic variants were identified across 289 NDD-associated genes. Almost 80% of the genes were reported in only one family, and 91% of the variants were non-recurrent. Autosomal recessive mode of inheritance accounted for approximately 86% of the total reported conditions, and 92% of the variants were identified in the homozygous state. Missense variants were the leading cause of NDDs (51%), followed by frameshift variants (22%), nonsense variants (18%), and splice-site or in-frame deletion variants (9%). Syndromic NDDs were more frequently reported than non-syndromic NDDs (79% versus 21%). Isolated intellectual disability was the most reported clinical phenotype, while *ASPM*, *WDR62*, and *TRAPPC9* were among the most frequently implicated genes. Most published families with available geographic information were recruited from Punjab and/or Khyber Pakhtunkhwa province. ClinVar annotations and ACMG/AMP-based assessments indicated that a substantial proportion of the reported variants were classified as pathogenic or likely pathogenic.

This review provides a nationwide overview of published genetic findings associated with NDDs in Pakistani families over an 18-year period. The marked predominance of autosomal recessive inheritance and homozygous variants reflects the important contribution of consanguinity to the genetic burden of NDDs in Pakistan. Whole-exome sequencing was the principal molecular diagnostic and gene-discovery approach. The identification of 424 variants across 289 genes highlights the extensive genetic and allelic heterogeneity of NDDs in Pakistan.

Key Message

Neurodevelopmental disorders in Pakistan are mostly driven by parental consanguinity.

Introduction

The brain of central nervous system (CNS) controls body functions and movements. It consists of nerves, the brain and spinal cord (1). Axon and neurons are the coordination parts in the brain of CNS. Any disruptions in it to cause neurodegenerative conditions may occur like Parkinson’s disease,

traumatic brain injury, Alzheimer's disease, stroke, multiple sclerosis and paraneoplastic disorders (2). Neurodevelopment refers to the biological processes underlying the nervous system formation and maturation. In humans, this process starts during the embryonic development third week with the formation of the neural tube (3). The disruption of different processes of brain growth and development may lead intellectual disability (ID), autism, idiopathic epilepsy, and schizophrenia like conditions called neurodevelopmental disorders (NDDs) (4). NDDs encompass conditions such as intellectual disability (ID), attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD) and motor disorders, communication, specific learning disorder and bipolar disorder (5). The most common featural condition in neurological disorders is ID which vary are mild to profound ID degrees based on intelligent quotient (IQ), phenotypic and behavioural variability (6).

Intellectual Disability (ID)

It is a condition to define a individual who has a restriction in mental functioning and abilities such as social skills, communication and self-care. These constraints, a kid will grow and learn extra sluggishly than a typical child. It is previously referred to as "mental retardation," a mental development disorder that consequences in a substantial limitation in particular mental capabilities, cognitive performance, learning ability, and function assistances when linked to people of the similar age, sex, and socioeconomic upbringing (7). According to the clinical definition of neurodevelopmental disorders (NDDs) of Diagnostic and Statistical Manual of Mental Disorders, 5th Edition DSM-5 defines ID as "A cluster of situations with start in the formative time considered by developmental defects that generate damages in personal, academic, social or academic," These impairments can be found in a variety of areas, including conversation, personal care, consciousness, and effective academic skills (8).

ID affects about 1% to 3% of people worldwide, while considerable geographical variances exist. Mild ID is thought to impact 0.7–1.3% of the general population, but severe and profound ID are thought to impact fewer than 0.5% ID is a significant public health issue that affects society and families, as well as health system financial burden, with direct expenses estimated a year at 43.3 billion euros in Europe. Developing countries' ID prevalence is higher than developed countries where it is not priority in the health, social and political policy. The bulk of profound ID is believed to take a single gene basis, whereas non-genetic as an environmental variables such as socio-cultural elements and contagions can lead to ID (9). Sheikh *et al*., (2018) conducted a study in the Lahore region of Punjab, Pakistan which showed 61% of males have ID. 44% of cases have moderate ID, 40% of cases have severe ID, mild ID cases 14%, and profound 2% ID cases. With ID, 54% of patients had a comorbid diagnosis (which includes epilepsy, extreme introvert range issue, oppositional disobedient turmoil, and consideration shortfall hyperactivity issue) (10). Overall, in Punjab, Pakistan prevalence of mild ID (46.7%), moderate ID (32.1%), severe ID (14.6%), and profound ID (6.7%) which males (56.82%) are more than females (43.17%). ID prevalence data is still missing from other provinces of Pakistan (11).

ID can be divided into two major classes independently (non-syndromic ID) or in conjunction through face dysmorphic traits, additional physical defects, and chronic (Syndromic ID, SID) (12). or a

combination of neuropsychological and/or neurocognitive issues, such as schizophrenia or seizure disorders, as well as neuromuscular characteristics, such as motor neuropathy, spastic paraplegia ataxia and spinal muscular atrophy (13). ID classes were based upon intelligence quotient (IQ) scores: mild (IQ 55–75, 85.0 percent of ID incidents), moderate (IQ 35–54, 10.0 percent of ID cases), severe (IQ 20–34, 3.5 percent of ID cases), and profound (IQ 1–20, 1.5 percent of ID cases) (7). ID diagnostic criteria usually involve mental functioning deficiencies acknowledged by medical assessment and average IQ testing; adaptive functioning shortages failing to fulfil neurodevelopment and sociocultural norms for individual self-reliance and self-responsibility, and emergence of deficiencies during the developmental process. The severity of ID is determined by an individual's adaptive functioning deficiencies in the intellectual, social, and practical domains, which dictates the level of assistance required (7). ID diagnostic criteria usually involve mental functioning deficiencies acknowledged by clinical assessment and standard IQ testing; adaptive functioning deficits failing to fulfil neurodevelopment and sociocultural norms for individual self-reliance and self-responsibility, and emergence of deficiencies through the developmental process. The brutality of ID is determined by an individual's adaptive functioning deficiencies trendy the intellectual, practical and social spheres, dictates the equal of assistance required (7). The identification of the genetic aetiology of ID is required for appropriate diagnosis, survival rate, and ailment prevention, and it plays a vital part in genetic counselling. Parents can be provided forecasting for coming pregnancies founded on the probability of illness reappearance and accessibility of a particular preimplantation (14). In the recent decade, technical improvements have discovered additional ID genes, shape new perspectives to molecular diagnostics and biological processes (15).

Mutations in genetic material, including DNA, RNA, or chromosomes, can lead to genetic diseases or altered phenotypes. That can be autosomal or X, Y chromosomal or mitochondrial pattern and its inheritance dominant or recessive way to affects the individuals in the pedigrees. Over 2800 ID genes, including major and candidate genes. There were more than1500 major ID genes, which cause about 1800 ID-related disease features, and over 1300 ID candidate genes, as per SysNDD (https://sysndd.dbmr.unibe.ch/). Most cases of monogenic ID are caused by single nucleotide variations (SNVs) besides minor insertions or deletions (indels), that code for proteins involved in essential genetic processes like neurogenesis, synaptogenesis, or synaptic plasticity. In 1975 the advancement of a sequencing technology such as Sanger sequencing (16). as well as additional automation and commercialization in the 1980s, stayed critical for the discovery of this type of mutation (17). It is difficult to diagnose, genetically counsel, and predict recurrence risk. In tri, tetra, and pentanucleotide dynamic mutations repeating sequences create a distinct subset of these mutations. In 1991, the first pathogenic mutations report of ID resulting from repeat expansions was published. ID harmful mutations induced by repetitive expansions were first reported in 1991. The discovery of a trinucleotide repetitive region, a CGG repeat tract, in the 5' untranslated regions of the FMRP translational regulator 1 gene (*FMR1*) associated with Fragile X syndrome (FXS) was reported in this work (18). Despite being diagnosed three decades ago, FXS remains the most prevalent cause of hereditary ID, and there is no effective therapy and little information on disease processes (19). Over 40 hereditary central nervous

system illnesses have been found so far (20, 21). Clinical genetic diagnostics tests and research methodologies to discover variants causing Mendelian or complex genetic characteristics for ID conditions have been transformed into fast sequencing technology. The utilization of familial genetic inheritance as an efficient way to find novel disease-causing mutations utilizing NGS platforms, and insilico tools were predicting the causative mutations for research or diagnostic test purposes (22).

Furthermore, well-known epigenetic disease processes such as DNA methylation and DNA imprinting do not follow a Mendelian inheritance pattern (23). ID, growth retardation, development, and metabolic problems have all been linked to imprinting illnesses, which are caused by disruptions in the dose, regulation and imprinting loci of genomic sequence (24). It's still difficult to find persistent and substantial methylation abnormalities in a group of unconnected but phenotypically comparable individuals (25). Imprinted genes have a monoallelic expression pattern that is determined by paternal origin (26). Prader-Willi (27), Angelman (28), Silver-Russell (29), Beckwith-Wiedemann (30), Temple (31), Kagami-Ogata syndromes (23), Transient Neonatal Diabetes (32), and Pseudohypoparathyroidism type 1B (33), are the eight well-known imprinting diseases to date.

In mitochondrial genome (mtDNA) pathogenic variations, which are also identified to be implicated in ID, are another type of diverse non-Mendelian genetic illness (34). Mitochondrial diseases are defined by a lack of oxidative phosphorylation, with an incidence of 2.9 instances per 100,000 people in adults and 9.6 cases per 100,000 people in children, correspondingly, due to mtDNA or nuclear abnormalitie (35). Around 1 in 200 healthy people has a mtDNA mutation with low levels of heteroplasmy, which has consequences for the progeny (36). Childhood-onset mitochondrial neurocognitive illnesses include Leigh syndrome, which is characterized by mutations in mtDNA and nuclear genes, and mtDNA Reduction disorder 4A (Alpers syndrome) (37).

Autism Spectrum Disorders (ASD)

Autism, also known as autism spectrum disorder (ASD) states to a heterogeneous group of multifactorial neurodevelopmental and neuropsychiatric disorders affect to children’s, characterized by substantial clinical heterogeneity (38). The term “spectrum” reflects the variability in symptom expression, with affected children involving differing levels of impairment, which ranges from mild to severe ASD (39, 40). The autism spectrum includes several related conditions, such as autistic disorder, Asperger syndrome, Rett syndrome and pervasive developmental disorder (40). ASD shows significant phenotypic heterogeneity, alongside numerous co-occurring disorders such as ID, Fragile X syndrome, ADHD, depression, anxiety, epilepsy, motor disorders, sleep disorders, digestive problems and dysmorphic features (41).

ASD prevalence has increased globally, presenting significant social, clinical, and familial challenges (42). The World Health Organization estimates that autism affects approximately 0.76% of children worldwide, the incident is higher in developed countries(43). The Centres for Disease Control and Prevention (CDC) reported that approximately 1 in 54 in the United States children were detected with

ASD during the dated from 2014 to 2016 (44). Notably, the prevalence of ASD varies across different ethnic populations. For example, estimates from South Korea suggest a prevalence of 2.64% (45), and in china around 12 per 10,000 individuals (46). A diagnosis of autism is often possible between 18 and 24 months, when behavioural symptoms emerge sufficiently to allow differentiation from typical development and other developmental disorders. Broader diagnostic criteria and increased awareness and understanding of ASD have substantially contributed to the growing number of reported diagnoses (47). ASD is diagnosed more recurrently in males than in females, with males showing a 4- to 7-fold greater risk; the causal reasons for this sex difference remain largely undecided (48). The current approach to diagnosing ASD is based on observing behavioural differences from predictable developmental patterns. These potentials of characteristic behaviour, however, are culturally dependent, so cultural norms can shape the expression and interpretation of ASD-related behaviour's (49). Autism spectrum disorder (ASD) is genetically heterogeneous, arising from both inherited sporadic and genomic alteration. A wide array of candidate genes has been identified as risk factor for ASD (50). ASD genetic studies have identified copy number variations and point mutations as key factors that contribute expressively to the heterogeneous genetic landscape of ASD (51). In current study of ASD trios of from Pakistan, Saudi Arabia and Iran, were known 84 candidate variants, of which the common (58%) were biallelic (52).

Attention-deficit/hyperactivity disorder (ADHD)

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent neurodegenerative condition marked by obstinate symptoms such as difficulty in attention, hyperactivity, and impulsive behavior. These difficulties can noticeably impact an individual's functioning across different stages of life. While ADHD was once considered exclusive to childhood, recent research indicates that the disorder often continues into adulthood (53). Psychiatric disorders frequently continue into adulthood, imposing considerable social and economic burdens. These include increased healthcare and societal costs, challenges in education and the welfare system, and implications for the justice system, as well as adverse effects on occupational performance, family and social relationships, and community engagement (54).

Globally, the prevalence of ADHD in children and teen-agers is estimated at approximately 7.6%, although rates vary considerably across different populations (55). Previous studies on epidemiological have described that the prevalence of adult ADHD among health students varies widely, range from 5.5% to 23.7% across different countries (56–58). A 2015 regional study published reported that 11.2% of school-aged children in Karachi were affected by ADHD (59). Diagnosing adult ADHD is often difficult due to symptom overlap with other psychiatric comorbidities. This complexity, coupled with limited recognition and screening, contributes to an underdiagnosed burden, leaving individuals without adequate support and interventions, and negatively affecting academic, occupational, and overall functioning (60). Although ADHD has a high heritability of 70–80%, its precise genetic architecture remains largely unknown. Genetic linkage studies were the first genome-wide approaches employed to identify DNA sequences transmitted within families affected by ADHD (61). ADHD has a polygenic

etiology, with multiple candidate genes involved in dopamine, noradrenaline, and serotonin pathways contributing to its complex traits (62). Key candidate genes associated with ADHD include those involved in dopaminergic and serotonergic signaling, such as *DAT1*, *DRD4*, *DRD5*, *5-HTT*, *HTR1B*, and *SNAP25* (63).

Motor Disorders

Motor disorders are a group of neurodegenerative ailments considered by degeneration of upper and lower motor neurons, including, spinal muscular atrophy, primary lateral sclerosis, hereditary spastic paraplegia, amyotrophic lateral sclerosis (ALS), and pseudobulbar palsy (64). Clinical manifestations often emerge during the first 20 years, causing significant functional impairment and a considerable burden on healthcare systems (65). Early symptoms typically include distal-to-proximal loss of stiffness and consciousness, primarily in the lower limbs, often accompanied by foot impairment and impaired fine motor skills in the hands (66). Motor disorders is recognized globally, affecting roughly 1 in every 3,300 people (67). Yet, in Pakistan consanguineous marriages are prevalent with 65% prevalence rate of these disorders (68). Investigations into the clinical and genetic features of motor disorders families in autosomal recessive have identified numerous genes associated with the disorder (69). Motor disorders genes are *GDAP1, MTMR2, NDRG1 SBF2, EGR2, PRX, FGD4, SH3TC2, PMP22, GJB1, MPZ, MED25, MFN2* and Fig. 4 (70).

Communication Disorders/Specific Learning Disorders

Communication disorders and specific learning disorders are key components of neurodevelopmental disorders (NDDs), a group of conditions that originate during early brain development and result in persistent difficulties across multiple functional domains. hese disorders typically present in childhood and interfere with language development, academic performance, and social participation (71). Unlike learning problems caused by environmental deprivation or acquired neurological injury, communication and learning disorders arise from intrinsic developmental abnormalities affecting neural systems involved in language processing and learning (72). Communication disorders and specific learning disorders frequently co-occur and share overlapping cognitive, neurobiological, and genetic risk factors. Language impairments are commonly observed in children with reading and writing difficulties, highlighting the close relationship between oral language development and academic skill acquisition. Neuroimaging and genetic studies suggest that both conditions involve atypical development of neural networks responsible for language processing, executive functioning, and learning, supporting their classification within the broader neurodevelopmental framework(73).

Population-based studies indicate that communication disorders, including language and speech impairments, affect approximately 5–10% of children globally, although prevalence estimates vary depending on diagnostic criteria, age group, and assessment methods (74). Specific learning disorders are similarly widespread, with global prevalence estimates ranging from 5–15% among school-aged

children. Dyslexia is the most common manifestation, accounting for the majority of diagnosed cases, while impairments in written expression and mathematics occur less frequently but often coexist with reading difficulties (75). Epidemiological data from diverse regions, including Europe, North America, and Asia, suggest comparable prevalence rates, although underdiagnosis remains a concern in low- and middle-income countries due to limited access to standardized assessment tools and specialized educational services (76).

Multiple genetic loci have been identified as contributors to the development of speech, language, and literacy skills (77). Among these, *FOXP2* is one of the most extensively studied genes and plays a critical role in speech motor control and language formulation, with rare pathogenic variants particularly associated with severe speech disorders such as childhood apraxia of speech (78). Several other genes including *CNTNAP2, DCDC2, KIAA0319, and DYX1C1* have been consistently associated with language impairment and reading-related difficulties, especially dyslexia (79, 80). These genes participate in fundamental neurodevelopmental mechanisms, such as neuronal migration, axonal pathfinding, synaptic organization, and modulation of cortical connectivity. Disruption of these processes may alter the formation and functioning of neural networks involved in language processing and academic learning, thereby increasing susceptibility to communication and specific learning disorders (81).

Next Generation Sequencing (NGS)

Next-Generation Sequencing (NGS), also called as high-throughput sequencing, is a authoritative tool that has transformed the field of genetic analysis. It allows scientists to sequence Nucleic acid fast and economically than previous (82). NGS can be used to sequence an entire genome. This is an important tool for understanding genetic variation and disease. By comparing the whole-genome sequences of different individuals, researchers can identify variants such as single nucleotide polymorphisms (SNPs), structural and insertions deletions (indels) that may be associated with disease (83). By providing fast, cost-effective, and high-resolution genetic data, NGS has significantly prolonged the study of genome and important for health and disease. It has become a fundamental tool in research, diagnostics, and personalized medicine. This newer method has greatly accelerated the pace of genetic discovery and have made it possible to study the genetics of complex traits and diseases in ways that were not possible before. Overall, genetic analysis is a critical tool in modern medicine and biology. It provides the opportunity for early detection, personalized treatment plans, improved medication response, and potential cures for genetic diseases. It also serves as a fundamental pillar of genetic and genomic research (83). PubMed search for " genetic intellectual disability by next generation sequencing" reveals the number of research publications per year over the past 12 years where NGS was used in genetic intellectual disability research (Fig. 1). Using bioinformatic pipeline for a typical NGS variant analysis procedure. Following clinical, physical, and early molecular studies, DNA samples are acquired. WGS is favoured for de novo mutations that are unknown or suspected, whereas most of the mendelian-type disorders investigated by WES. NGS raw reads is pre-processed for removal of low-quality sequences

prior to variant analysis. Following alignment to a reference genome or chromosome, alignment sorting, duplication removal, variation calling, and annotation are all performed (83).

Methods

This systematic review was conducted in accordance with the Declaration of Helsinki and in adherence with the PRISMA guidelines. The inclusion criteria encompassed genetic studies involving Pakistani families affected by syndromic or non-syndromic hereditary neurological disorders published between 2006 and 2024.

A comprehensive literature search was conducted using major databases and academic search platforms, including PubMed (NCBI), Google Scholar, ResearchGate, and Web of Science. Relevant articles were identified using combinations of keywords such as “neurological disorders in Pakistani families,” “hereditary neurological disorders,” “psychiatric disorders,” “epilepsy,” “intellectual disability,” “Pakistani mutations,” “whole-exome sequencing,” “attention-deficit/hyperactivity disorder,” “developmental delay,” “motor disorders,” “communication disorders,” “specific learning disorders,” “microcephaly,” “Huntington’s disease,” and “autism spectrum disorder.”

All retrieved articles were rigorously screened. Studies that did not report a genetic variant or corresponding cDNA-level change were excluded. Eligible studies included those investigating neurodevelopmental disorders, neurodegenerative disorders, and intellectual disability.

Relevant demographic, clinical, and genetic data were systematically extracted from the selected studies and organized in tabular form using Microsoft Excel. The extracted data were manually curated to ensure accuracy, completeness, and consistency.

VariantValidator was used to verify and standardize the genomic nomenclature of all reported variants. Detailed variant annotations, including cDNA- and protein-level consequences, gene symbols, transcript and protein identifiers, genomic coordinates, inheritance patterns, and allele frequencies, were obtained from major genomic databases, including Ensembl, the UCSC Genome Browser, OMIM, and gnomAD.

All identified variants were further assessed using clinically relevant databases, including ClinVar, the Human Gene Mutation Database, and other online tools e.g., VarSome, to determine their reported clinical significance. The variant pathogenicity were reclassified using the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines using VarSome with manual curation.

In addition, the potential pathogenic impact of missense variants was evaluated using multiple in-silico prediction tools, including SIFT, CADD, PROVEAN, PolyPhen-2, MutationTaster, MutPred, LRT, FATHMM, REVEL, DANN, AlphaMissense, and MutationAssessor.

Results

General Characteristics

This review included 145 research articles published between May 2006 and May 2024 that investigated the genetic basis of neurodevelopmental disorders (NDDs) in Pakistani families. Overall, the number of publications increased steadily over time, with the highest annual output observed during the last five years (Fig. 1A).

Across the included studies, 537 Pakistani families comprising 1,664 individuals (815 affected individuals and 849 unaffected relatives or healthy controls) underwent genetic investigation. Most families were consanguineous (85%). Geographic information was available for 58% of families, whereas the remaining 42% had no reported geographic origin. Among families with known geographic origin, most were from Punjab (43%), followed by Khyber Pakhtunkhwa (25%), Azad Jammu and Kashmir (AJK; 6%), Sindh (5%), and other regions (21%) (Fig. 3).

WES was the predominant genetic testing approach, accounting for 85% of studies, followed by targeted sequencing (11%), WES combined with targeted sequencing (3%), and whole-genome sequencing (WGS; 1%) (Fig. 2c).

Overall, 79% of cases were classified as syndromic NDDs, whereas 21% were classified as non-syndromic (Fig. 3). The reported cases encompassed five major clinical categories: intellectual disability (ID), autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), motor disorders (MD), and communication disorders/specific learning disorders (CD/SLD). Among the 537 families, isolated ID was the most frequently reported phenotype (218/537, 40%), followed by ASD (41/537, 8%), MD (25/537, 5%), and ADHD (4/537, 1%). The remaining 249 families (46%) exhibited diverse clinical presentations involving multiple NDD phenotypes and were therefore classified as a mixed group (Fig. 3).

Genetic Characteristics

Across all molecularly diagnosed families, 424 distinct variants were identified in 289 genes previously known to cause neurodevelopmental disorder (NDDs) (Supplementary Table 1). Of these genes, 230 (80%) were implicated in a single family, whereas 59 (20%) were implicated in at least two families. Mutations in *ASPM* and *WDR62* were among the leading causes of NDDs, accounting for 13% and 5%, respectively. While a significant proportion (57.5%) of genes carried only one variant in the published reports, genes carrying multiple variants (42.5%) were also reported in this study (Fig. 2a). Next, we evaluated the recurrence of individual (same) variants across multiple reports. Variants reported in more than one study were classified as recurrent; however, the available data did not allow us to determine whether patients carrying the same variant were biologically related. Of the 424 distinct variants identified, 386 (91%) were reported only once, whereas 38 (9%) were reported multiple times across independent studies.

In terms of inheritance, as expected autosomal recessive mode of inheritance was accounted for 86% of all reported patients followed by autosomal dominant (3%), and X-linked (2%). However, in 9% cases the

inheritance pattern was unknown thus excluded from the analysis (Fig. 2b). Among the AR cases, 92% of the PLP variants were found in homozygous state and 8% cases were in compound heterozygous state.

Collectively, missense variants were predominant (51%) followed by frameshift (22%), stop-gain/start loss (18%), splicing (6%), and in-frame deletions (3%). Fifty-two percent (221/424) of the variants reported in this review were found on the ClinVar database including 70% as PLP, 18% as VUS, and 12% as BLB or conflicting. Likewise, as per ACMG criteria, 47% variants enlisted here were PLP, 31% were VUS, and 22% were BLB (Fig. 3).

We further investigated all reported missense variants (n = 216) for their predicted pathogenicity by utilizing databases such as ACMG and ClinVar (Supplementary Table 2). As per ACMG criteria, 25 missense variants were PLP, 103 were VUS, and 88 were BLB. The later 88 BLB missense variants were further evaluated using online in-silico tools/databases like MutScore, MetaRNN, CADD, REVEL, phyloP100, DANN, LRT, Mutation Assessor, Mutation Taster, Mutpred, PolyPhen-2, PROVEAN, SIFT, HGMD, and gnomAD (Supplementary Table 3). Upon querying all missense variants in the ClinVar database, we have found that 111 variants were enlisted in the ClinVar whereas 105 variants were not registered. Of those 111 variants that were registered in the ClinVar, 54 were PLP, 34 were VUS, and 23 were BLB (including variants with conflicting interpretation). The distribution of ACMG classifications for all missense substitutions by wild-type residues is shown in Supplementary data. Our analysis showed that most PLP substitutions occurred in serine (8), threonine (4), phenylalanine (3), and valine (3) residues, suggesting that changes in these specific amino acids may negatively affect protein function. On the other hand, VUS were the most frequently observed substitutions for glycine (15), glutamine (14), serine (14), and tryptophan (12). Lastly, BLB variants were enriched in alanine (6), glycine (8) and proline residues (8).

We performed gene intersection analysis to dissect overlapping and non-overlapping genes across seven sub-populations of Pakistan. Of the assembled 289 mutated genes, geographical affiliation was explicitly mentioned for 170 genes in the reports; however, no geographical origin was associated, by the authors, for the remaining 119 genes. Overall, 105 distinct genes were reported from Punjab, 60 genes from KPK, 14 genes from AJK, 9 genes from Sindh, 5 genes from Baluchistan, 4 genes from Islamabad, and 2 genes from Gilgit Baltistan (GB). Altogether, 147 genes were (unique) specific to a certain population whereas 23 genes overlapped between two or more geographically sub-populations (Fig. 4).

Seventy-eight percent (82/105) genes were solely reported from the Punjab province whereas the remaining twenty-two percent genes (23/105) were shared between Punjab and other geographical localities. Likewise, 70% (42/60) genes in KPK were unique while 30% (18/60) were shared by other sub-populations. AJK had 71% (10/14) unique and 29% (4/14) shared genes. Sindh had 67% (6/9) unique and 33% (3/9) shared genes, Baluchistan had 60% (3/5) unique and 40% (2/5) shared genes, Islamabad had 75% (3/4) unique and 25% (1/4) shared genes. Lastly, Gilgit Baltistan had equal proportion of shared and unique genes (1 unique, 1 shared) To sum up, highest number of unique genes were found in Punjab (n = 82), followed by KPK (n = 42), AJK (n = 10), Sindh (n = 6), Baluchistan (n = 3), Islamabad (n = 3), and Gilgit

Baltistan (n = 1). Of the 289 total genes reported here, 214 were exclusively associated to a specific NDDs phenotype while 75 were shared across different clinical sub-types of NDDs. Isolated ID was the most genetically heterogeneous phenotype with 164 associated genes solely accounting for ID, followed by ASD (38 genes), MD (9 genes), and ADHD (3 gene). Surprisingly, no unique gene was found to cause CD/SLDs exclusively. However, 46 genes across different NDDs types were found to overlap clinically with CD/SLD categories. Interestingly, mutations in three genes including *PCNT*, *NARS1*, and *FA2H* were reported to cause an array of NDDs phenotypes including ADHD, MD, ID, ASD and CD/SLD (Fig. 5). Generally, pathological changes in *ASPM* and *WDR62* were the most leading causes of NDDs in Pakistan that collectively accounted for over 17% (73/424) of all the reported variants in this study. Individually, *ASPM* carried 54 different pathogenic variants including 24 nonsense, 23 frameshift, 5 missense, 1 splicing, and 1 synonymous SNV whereas *WDR62* carried, in total, 19 distinct variants comprising 6 frameshift, 6 missense, 3 nonsense, 2 splicing and 2 in-frame deletions (Fig. 6). Variants inside coding regions of *ASPM* and *WDR62* genes are shown in Fig. 7.

Discussion

Neurodevelopmental disorders (NDDs) are a heterogenous group of prevalent genetic disorders that primarily result from underdevelopment of the central nervous system early in life. NDDs affect ~ 2–4% of the world's population leaving life-long impacts on patients and their families (84–86). In the Asia-Pacific region, NDDs affect ~ 7.5 per 1,000 children (87). Similarly, Pakistan is leading the world in terms of burden of hereditary disorders showing regional variation likely owing to fluctuating rates of consanguinity and endogamy (88–90). As expected, 85% NDDs cases reported in this study belonged to consanguineous families. Thus, regardless of category of monogenic disorders, the prevalence of parental consanguinity remained always high in all published Pakistani cohorts. For example, parental consanguinity rates were found to be approximately 70% in inherited retinal dystrophies (91), 74% in blood disorders (92), 82% in skeletal disorders (93), 87% in deafness (94), and 91% in NDDs (95). Thus, identification of disease-causing variants by consanguinity driven homozygosity mapping could greatly reduce cost and improve patients care.

Although genetic studies of neurodevelopmental disorders from Pakistan have been reported since the mid-2000s, early investigations were largely limited to cytogenetic and targeted gene approaches (96). Over the years, the development of advanced technologies (WES,WGS and long-read sequencing) and improved computational workflows, high accuracy and throughput has enabled full coverage of complex genomic regions, non-coding sequences and structural variants, facilitating the identification of variants that were previously difficult to detect. These and other reasons may contribute to the recent surge in publications related to NDDs as observed in this review (97–100). The disproportionately higher NDDs cases reported from Punjab and/or KPK provinces of Pakistan could be attributed to high population density, prevalent consanguineous marriages and the availability of clinical genetics services which strongly correlated with increase prevalence of homozygous recessive condition (101, 102).

Further, integrating genetic testing into clinical setting has proved key in diagnosing patients with rare diseases, increasing the diagnostic rate to 50.8% (103, 104). Thus, genetic studies from Pakistan have played an important role in genes discovery across wide range of inherited conditions. Previous reports from Pakistan have documented a large number of genes associated with Mendelian disorders, including inherited retinal diseases (87 genes, 277 variants) (91), neurodevelopmental disorders (143 genes, 188 variants) (95), dermatological disorders (19 genes) (105–108) and hereditary deafness (51 genes) (109).

Pakistan's high consanguinity rates have led to numerous natural human knockouts, with whole-exome sequencing of 10,503 individuals revealing ~ 49,138 rare loss-of-function mutations, including complete knockout of 1,317 genes, which likely contributes to the high burden of genetic disorders (110). Our study identified 289 genes harboring 424 variants in NDDs. Importantly, 91% of variants identified in this review were rare, as they were reported only once to underscore the significant contribution of rare, high-impact mutations to NDDs etiology.

To explore the regional distribution of genes associated with NDD in Pakistan, we analyzed genes distribution across different provinces of Pakistan. Previous NDDs genetic research on overall Pakistani population has largely been limited. The multiethnic composition of our dataset enabled the analysis of genes both within and across geographic regions of Pakistan. Certain variants were region-specific as rare, whereas others were shared across different regions as common. This enabled the identification of risk genes harboring rare or common variants that contribute to NDDs within specific populations or across several regions (111). Further, founder Mutations Punjab and KPK shared the highest numbers of genes responsible for disease phenotypes. Both provinces may reflect a combination of demographic, cultural and genetic factors. These regions have high rate of consanguineous marriage, intra-family union and shared tribal and migration histories. This may facilitate the founder alleles across both regions (112, 113). Such is the case in Pakistan's as several founder mutations have been identified in various genes. These include *ASPM* (NM_018136: c.3978G > A), *FA2H* (NM_024306: c.159_176del) and *MBOAT7* (NM_024298: c.758_778del). While some disease alleles may vanish from the genetic pool due to natural selection, variants with founder effects may survive for a long time, thereby enhancing the prevalence of some rare diseases. These are predominantly autosomal recessive diseases but can also be autosomal dominant traits with late-onset or mild phenotypes [PMID: 38922202].

ASPM and *WDR62* are key regulators of centrosome and centriole function in neural progenitor cells. *ASPM* aids in recruitment of centrosomal proteins during centriole duplication, while WDR62 stabilizes the mitotic spindle and organizes spindle poles. Disruption of these pathways impairs neural progenitor proliferation, leading to reduced brain size characteristics of primary microcephaly (114). Previous reports from Pakistan and broader Asian populations shows these two genes represent the predominant genetic contributors to autosomal recessive primary microcephaly (115, 116). Consistent with global and regional data (115, 116) our findings show *ASPM* and *WDR62* are among the most mutated genes in our study which reported in all geographical regions of Pakistan.

NDDs are clinically heterogeneous group of disorders that includes ID, ASD, ADHD, MD, and CD/SLD. Of them, ID affect 1–3% of the population globally (117). In our study, we found ID the prevalent (40%) class of NDDs. Regardless of their diverse clinical phenotypes, recent studies have confirmed that some genes are shared across multiple NDDs subclasses, reflecting overlapping biological pathways (118). *PCNT* encodes the centrosomal protein pericentrin, which regulates cell division; mutations in *PCNT* can lead to microcephaly and neurodevelopmental defects (96). *NARS1* encodes asparaginyl-tRNA synthetase, and loss of its function reduces radial glial cell proliferation during cortical development, leading to microcephaly and neurodevelopmental delay (119). *FA2H* encodes fatty acid 2-hydroxylase, an enzyme required for synthesis of hydroxylated fatty acids that are essential components of myelin; loss of its function leads to defective myelin formation and demyelination in neurodevelopmental disorders (120). Our findings show that 79% of syndromic cases which are associated with genes involved in centrosome, spindle, and cilia-related pathways, including *ASPM, WDR62, CEP135, CEP152*, and *PCNT*, while only five genes (*ARL13B, CC2D2A, TMEM67, EXOC8, and MKKS*) represent strictly established ciliopathies by gene panel. In our analysis, we found that *PCNT, NARS1*, and *FA2H* overlap across all examined NDDs subclasses, indicating shared biological mechanisms influencing neurodevelopment. The molecular interaction of these genes may underly the phenotypic heterogeneity that may impact various developmental pathways (118).

Conclusion

We provide a comprehensive compendium of the genetic variants causing a range of neurodevelopmental disorders (NDDs) in Pakistani families over an 18-year period (2006–2024). Our analysis demonstrates a pronounced predominance of autosomal recessive inheritance, largely driven by high rates of parental consanguinity, resulting in widespread homozygosity of rare pathogenic variants. Intellectual disability emerged as the most frequently reported phenotype. Whole-exome sequencing was the preferred molecular diagnostic approach followed in majority of cases. Notably, *ASPM, WDR62* and *TRAPPC9* were the most recurrently implicated genes, while the majority of variants and genes were family or population specific. Geographic stratification further revealed substantial regional genetic diversity, particularly within Punjab and Khyber Pakhtunkhwa provinces. Collectively, these findings underscore the remarkable genetic and allelic heterogeneity of NDDs in Pakistan and highlight the critical need for population-specific variant annotation, functional validation and expanded genomic studies to improve molecular diagnosis and genetic counselling.

Limitations

This review is based only on previously published studies and online databases; therefore, it cannot prove a direct cause-and-effect relationship between genetic variants and diseases. Some relevant studies may have been missing, and in many articles important information such as patient details, variant classification, or prediction analyses was not fully reported, which may affect the results. In addition, most data came from a few regions of Pakistan, so the findings may not fully represent all

populations, and older cases could not be included because whole-exome sequencing has been used in Pakistan only in recent years.

Abbreviations

NDDs	Neurodevelopmental Disorders
ACMG	American College of Medical Genetics and Genomics
ID	Intellectual Disability
MD	Motor Disorders
ASD	Autism Spectrum Disorder
ADHD	Attention-Deficit/Hyperactivity Disorder
CD/SLD	Communication Disorder/ Specific Learning Disorder
CNS	Central Nervous System
IQ	Intelligent Quotient
SNVs	Single Nucleotide Variations
Indels	Insertions or Deletions
FXS	Fragile X Syndrome
mtDNA	Mitochondrial DNA
CDC	Centers for Disease Control
ALS	Amyotrophic Lateral Sclerosis
NGS	Next-Generation Sequencing
SNPs	Single Nucleotide Polymorphisms
NDGDs	Neurodegenerative Disorders
AJK	Azad Jammu and Kashmir
KPK	Khyber Pakhtunkhwa
WES	Whole Exome Sequencing

WGS	Whole Genome Sequencing
VUS	Variant of Uncertain Significance
BLB	Benign Likely Benign
PLP	Pathogenic Likely Pathogenic
GB	Gilgit Baltistan

Declarations

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Data availability statement

All the data relevant to this manuscript have either been mentioned in this manuscript or could be available in the supplementary files of this manuscript.

Author Contributions

F.A., S.Z. and A.U.R. contributed equally to this study and played major roles in data collection, analysis, interpretation of published studies, and manuscript preparation. S.U., H.U.R and A.A. contributed to figure preparation and data presentation. A.H. and H.K. contributed to writing the introduction section. K.K., M.N., I.U., M.U., A.R. and A.M. contributed to the search for literature, collection of published data, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript. A.U.R. supervised this study.

References

1. Seraji-Bzorgzad N, Paulson H, Heidebrink J. Neurologic examination in the elderly. Handb Clin Neurol. 2019;167:73–88.
2. Amor S, Peferoen LA, Vogel DY, Breur M, van der Valk P, Baker D, et al. Inflammation in neurodegenerative diseases–an update. Immunology. 2014;142(2):151–66.
3. Stiles J, Jernigan TL. The basics of brain development. Neuropsychol Rev. 2010;20(4):327–48.
4. Mitchell KJ. The genetics of neurodevelopmental disease. Curr Opin Neurobiol. 2011;21(1):197–203.
5. Niemi MEK, Martin HC, Rice DL, Gallone G, Gordon S, Kelemen M, et al. Common genetic variants contribute to risk of rare severe neurodevelopmental disorders. Nature. 2018;562(7726):268–71.
6. Wan RP, Liu ZG, Huang XF, Kwan P, Li YP, Qu XC, et al. YWHAZ variation causes intellectual disability and global developmental delay with brain malformation. Hum Mol Genet. 2023;32(3):462–72.
7. Grzegorzewska AM, Wiglusz MS, Cubała WJ, Jakuszkowiak-Wojten K, Włodarczyk A, Szarmach J. Dysphoria and Irritability-Diagnostic Pitfalls in the Assessment of Interictal Dysphoric Disorder in Epilepsy. J Clin Med. 2021;10(19).
8. Cheroni C, Caporale N, Testa G. Autism spectrum disorder at the crossroad between genes and environment: contributions, convergences, and interactions in ASD developmental pathophysiology. Mol Autism. 2020;11(1):69.
9. Patel DR, Cabral MD, Ho A, Merrick J. A clinical primer on intellectual disability. Transl Pediatr. 2020;9(Suppl 1):S23–35.
10. Sheikh MH, Ashraf S, Imran N, Hussain S, Azeem MW. Psychiatric Morbidity, Perceived Stress and Ways of Coping Among Parents of Children With Intellectual Disability in Lahore, Pakistan. Cureus. 2018;10(2):e2200.
11. Song C, Han T, Hu L, Shao N, Wang Z, Jin Y, et al. Early developmental levels of children with autism spectrum disorder with different adaptive behaviors: a retrospective analysis. J Zhejiang Univ Sci B. 2024;25(11):1031–8.
12. Kochinke K, Zweier C, Nijhof B, Fenckova M, Cizek P, Honti F, et al. Systematic Phenomics Analysis Deconvolutes Genes Mutated in Intellectual Disability into Biologically Coherent Modules. Am J Hum Genet. 2016;98(1):149–64.
13. Chiurazzi P, Pirozzi F. Advances in understanding - genetic basis of intellectual disability. F1000Research. 2016;5.
14. Rauch A, Hoyer J, Guth S, Zweier C, Kraus C, Becker C, et al. Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. Am J Med Genet Part A. 2006;140(19):2063–74.
15. Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. Nat Rev Genet. 2016;17(1):9–18.
16. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol. 1975;94(3):441–8.

17. Ansorge W, Sproat BS, Stegemann J, Schwager C. A non-radioactive automated method for DNA sequence determination. J Biochem Biophys Methods. 1986;13(6):315–23.
18. Verkerk AJ, Pieretti M, Sutcliffe JS, Fu YH, Kuhl DP, Pizzuti A, et al. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell. 1991;65(5):905–14.
19. Ciaccio C, Fontana L, Milani D, Tabano S, Miozzo M, Esposito S. Fragile X syndrome: a review of clinical and molecular diagnoses. Ital J Pediatr. 2017;43(1):39.
20. Paulson H. Repeat expansions in leukoencephalopathy. Ann Neurol. 2019;86(6):809–11.
21. Seixas AI, Loureiro JR, Costa C, Ordóñez-Ugalde A, Marcelino H, Oliveira CL, et al. A Pentanucleotide ATTTC Repeat Insertion in the Non-coding Region of DAB1, Mapping to SCA37, Causes Spinocerebellar Ataxia. Am J Hum Genet. 2017;101(1):87–103.
22. Kanzi AM, San JE, Chimukangara B, Wilkinson E, Fish M, Ramsuran V, et al. Next Generation Sequencing and Bioinformatics Analysis of Family Genetic Inheritance. Front Genet. 2020;11:544162.
23. Kagami M, Sekita Y, Nishimura G, Irie M, Kato F, Okada M, et al. Deletions and epimutations affecting the human 14q32.2 imprinted region in individuals with paternal and maternal upd(14)-like phenotypes. Nat Genet. 2008;40(2):237–42.
24. Reik W, Walter J. Genomic imprinting: parental influence on the genome. Nat Rev Genet. 2001;2(1):21–32.
25. Hannon E, Gorrie-Stone TJ, Smart MC, Burrage J, Hughes A, Bao Y, et al. Leveraging DNA-Methylation Quantitative-Trait Loci to Characterize the Relationship between Methylomic Variation, Gene Expression, and Complex Traits. Am J Hum Genet. 2018;103(5):654–65.
26. Peters J. The role of genomic imprinting in biology and disease: an expanding view. Nat Rev Genet. 2014;15(8):517–30.
27. Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. Genet medicine: official J Am Coll Med Genet. 2012;14(1):10–26.
28. Williams CA, Driscoll DJ, Dagli AI. Clinical and genetic aspects of Angelman syndrome. Genet medicine: official J Am Coll Med Genet. 2010;12(7):385–95.
29. Wakeling EL, Amero SA, Alders M, Bliek J, Forsythe E, Kumar S, et al. Epigenotype-phenotype correlations in Silver-Russell syndrome. J Med Genet. 2010;47(11):760–8.
30. Weksberg R, Shuman C, Beckwith JB. Beckwith-Wiedemann syndrome. Eur J Hum genetics: EJHG. 2010;18(1):8–14.
31. Ioannides Y, Lokulo-Sodipe K, Mackay DJ, Davies JH, Temple IK. Temple syndrome: improving the recognition of an underdiagnosed chromosome 14 imprinting disorder: an analysis of 51 published cases. J Med Genet. 2014;51(8):495–501.
32. Docherty LE, Kabwama S, Lehmann A, Hawke E, Harrison L, Flanagan SE, et al. Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype-phenotype correlation in

an international cohort of patients. Diabetologia. 2013;56(4):758–62.
33. Bastepe M. The GNAS locus and pseudohypoparathyroidism. Adv Exp Med Biol. 2008;626:27–40.
34. Craven L, Alston CL, Taylor RW, Turnbull DM. Recent Advances in Mitochondrial Disease. Annu Rev Genom Hum Genet. 2017;18:257–75.
35. Gorman GS, Schaefer AM, Ng Y, Gomez N, Blakely EL, Alston CL, et al. Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Ann Neurol. 2015;77(5):753–9.
36. Elliott HR, Samuels DC, Eden JA, Relton CL, Chinnery PF. Pathogenic mitochondrial DNA mutations are common in the general population. Am J Hum Genet. 2008;83(2):254–60.
37. Lake NJ, Compton AG, Rahman S, Thorburn DR. Leigh syndrome: One disorder, more than 75 monogenic causes. Ann Neurol. 2016;79(2):190–203.
38. Khalid M, Raza H, Tejwani TMDPJL, Sami L. A, Genetic Risk of Autism Spectrum Disorder in a Pakistani Population. Genes (Basel). 2020;11(10).
39. Jokiranta-Olkoniemi E, Cheslack-Postava K, Sucksdorff D, Suominen A, Gyllenberg D, Chudal R, et al. Risk of Psychiatric and Neurodevelopmental Disorders Among Siblings of Probands With Autism Spectrum Disorders. JAMA psychiatry. 2016;73(6):622–9.
40. Robert C, Pasquier L, Cohen D, Fradin M, Canitano R, Damaj L et al. Role of Genetics in the Etiology of Autistic Spectrum Disorder: Towards a Hierarchical Diagnostic Strategy. Int J Mol Sci. 2017;18(3).
41. Shankar R, Perera B, Thomas RH. Epilepsy, an orphan disorder within the neurodevelopmental family. J Neurol Neurosurg Psychiatry. 2020;91(12):1245–7.
42. Lord C, Elsabbagh M, Baird G, Veenstra-Vanderweele J. Autism spectrum disorder. Lancet (London England). 2018;392(10146):508–20.
43. Zeidan J, Fombonne E, Scorah J, Ibrahim A, Durkin MS, Saxena S, et al. Global prevalence of autism: A systematic review update. Autism research: official J Int Soc Autism Res. 2022;15(5):778–90.
44. Wang X, Wu D, Luo T, Fan W, Li J. Impact of interaction between individual genomes and preeclampsia on the severity of autism spectrum disorder symptoms. Zhong nan da xue xue bao Yi xue ban = Journal of Central South University Medical sciences. 2024;49(8):1187–99.
45. Kim YS, Leventhal BL, Koh YJ, Fombonne E, Laska E, Lim EC, et al. Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry. 2011;168(9):904–12.
46. Sun X, Allison C, Matthews FE, Sharp SJ, Auyeung B, Baron-Cohen S, et al. Prevalence of autism in mainland China, Hong Kong and Taiwan: a systematic review and meta-analysis. Mol Autism. 2013;4(1):7.
47. Rosen NE, Lord C, Volkmar FR. The Diagnosis of Autism: From Kanner to DSM-III to DSM-5 and Beyond. J Autism Dev Disord. 2021;51(12):4253–70.
48. Werling DM, Geschwind DH. Understanding sex bias in autism spectrum disorder. Proc Natl Acad Sci USA. 2013;110(13):4868–9.

49. Norbury CF, Sparks A. Difference or disorder? Cultural issues in understanding neurodevelopmental disorders. Dev Psychol. 2013;49(1):45–58.
50. Hodges H, Fealko C, Soares N. Autism spectrum disorder: definition, epidemiology, causes, and clinical evaluation. Transl Pediatr. 2020;9(Suppl 1):S55–65.
51. Mamidala MP, Kalikiri MK, Praveen Kumar PT, Rajesh N, Vallamkonda OR, Rajesh V. Consanguinity in India and its association with autism spectrum disorder. Autism research: official J Int Soc Autism Res. 2015;8(2):224–8.
52. Khan H, Harripaul R, Mikhailov A, Herzi S, Bowers S, Ayub M, et al. Biallelic variants identified in 36 Pakistani families and trios with autism spectrum disorder. Sci Rep. 2024;14(1):9230.
53. Song P, Zha M, Yang Q, Zhang Y, Li X, Rudan I. The prevalence of adult attention-deficit hyperactivity disorder: A global systematic review and meta-analysis. J Glob Health. 2021;11:04009.
54. Barican JL, Yung D, Schwartz C, Zheng Y, Georgiades K, Waddell C. Prevalence of childhood mental disorders in high-income countries: a systematic review and meta-analysis to inform policymaking. Evid Based Ment Health. 2022;25(1):36–44.
55. Salari N, Ghasemi H, Abdoli N, Rahmani A, Shiri MH, Hashemian AH, et al. The global prevalence of ADHD in children and adolescents: a systematic review and meta-analysis. Ital J Pediatr. 2023;49(1):48.
56. Atwoli L, Owiti P, Manguro G, Ndambuki D. Attention deficit hyperactivity disorder symptom self-report among medical students in Eldoret, Kenya. Afr J Psychiatry (Johannesbg). 2011;14(4):286–9.
57. Christiansen H, Kis B, Hirsch O, Matthies S, Hebebrand J, Uekermann J, et al. German validation of the Conners Adult ADHD Rating Scales (CAARS) II: reliability, validity, diagnostic sensitivity and specificity. Eur psychiatry: J Association Eur Psychiatrists. 2012;27(5):321–8.
58. Mason A, Rapsey C, Sampson N, Lee S, Albor Y, Al-Hadi AN, et al. Prevalence, age-of-onset, and course of mental disorders among 72,288 first-year university students from 18 countries in the World Mental Health International College Student (WMH-ICS) initiative. J Psychiatr Res. 2025;183:225–36.
59. Ibrahim M. Bibliometric analysis of the Journal of Pakistan Medical Association form 2009 to 2013. J Pak Med Assoc. 2015;65(9):978–83.
60. Anbarasan D, Kitchin M, Adler LA. Screening for Adult ADHD. Curr Psychiatry Rep. 2020;22(12):72.
61. Zhou K, Dempfle A, Arcos-Burgos M, Bakker SC, Banaschewski T, Biederman J, et al. Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. Am J Med Genet Part B Neuropsychiatric genetics: official publication Int Soc Psychiatric Genet. 2008;147b(8):1392–8.
62. Verlaet AAJ, Maasakkers CM, Hermans N, Savelkoul HFJ. Rationale for Dietary Antioxidant Treatment of ADHD. Nutrients. 2018;10(4).
63. Gizer IR, Ficks C, Waldman ID. Candidate gene studies of ADHD: a meta-analytic review. Hum Genet. 2009;126(1):51–90.

64. Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. Nat reviews Disease primers. 2017;3:17085.
65. Yasha TC, Sharma S, Gayathri N, Nandeesh NB. Hereditary neuropathies: Genetics and utility of nerve biopsy. Indian J Pathol Microbiol. 2022;65(Supplement):S318–28.
66. Visser HJ, Wolfe J, Kouri R, Aviles R. Neurologic Conditions Associated with Cavus Foot Deformity. Clin Podiatr Med Surg. 2021;38(3):323–42.
67. Finsterer J, Löscher WN, Wanschitz J, Iglseder S. Orphan Peripheral Neuropathies. J Neuromuscul Dis. 2021;8(1):1–23.
68. Dubourg O, Azzedine H, Verny C, Durosier G, Birouk N, Gouider R, et al. Autosomal-recessive forms of demyelinating Charcot-Marie-Tooth disease. Neuromolecular Med. 2006;8(1–2):75–86.
69. Tazir M, Bellatache M, Nouioua S, Vallat JM. Autosomal recessive Charcot-Marie-Tooth disease: from genes to phenotypes. J peripheral Nerv system: JPNS. 2013;18(2):113–29.
70. Geroldi A, Ponti C, Mammi A, Patrone S, Gotta F, Trevisan L, et al. Early Onset Inherited Peripheral Neuropathies: The Experience of a Specialized Referral Center for Genetic Diagnosis Achievement. Pediatr Neurol. 2024;154:4–8.
71. Vernes SC, Fisher SE. Unravelling neurogenetic networks implicated in developmental language disorders. Biochem Soc Trans. 2009;37(Pt 6):1263–9.
72. Newbury DF, Monaco AP. Genetic advances in the study of speech and language disorders. Neuron. 2010;68(2):309–20.
73. Fujita E, Tanabe Y, Momoi MY, Momoi T. Cntnap2 expression in the cerebellum of Foxp2(R552H) mice, with a mutation related to speech-language disorder. Neurosci Lett. 2012;506(2):277–80.
74. Snowling MJ, Hulme C. Interventions for children's language and literacy difficulties. Int J Lang communication disorders. 2012;47(1):27–34.
75. Graham SA, Fisher SE. Decoding the genetics of speech and language. Curr Opin Neurobiol. 2013;23(1):43–51.
76. Peterson RL, Pennington BF. Developmental dyslexia. Lancet (London England). 2012;379(9830):1997–2007.
77. Snijders Blok L, Rousseau J, Twist J, Ehresmann S, Takaku M, Venselaar H, et al. CHD3 helicase domain mutations cause a neurodevelopmental syndrome with macrocephaly and impaired speech and language. Nat Commun. 2018;9(1):4619.
78. Morgan A, Fisher SE, Scheffer I, Hildebrand M. FOXP2-Related Speech and Language Disorder. In: Adam MP, Bick S, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews(®). Seattle (WA): University of Washington, Seattle Copyright © 1993–2026, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
79. Zhang J, Cai F, Lu R, Xing X, Xu L, Wu K, et al. CNTNAP2 intracellular domain (CICD) generated by γ-secretase cleavage improves autism-related behaviors. Signal Transduct Target therapy.

2023;8(1):219.
80. Zhong R, Yang B, Tang H, Zou L, Song R, Zhu LQ, et al. Meta-analysis of the association between DCDC2 polymorphisms and risk of dyslexia. Mol Neurobiol. 2013;47(1):435–42.
81. Deng KG, Zhao H, Zuo PX. Association between KIAA0319 SNPs and risk of dyslexia: a meta-analysis. J Genet. 2019;98(1).
82. Slatko BE, Gardner AF, Ausubel FM. Overview of Next-Generation Sequencing Technologies. Curr Protoc Mol Biol. 2018;122(1):e59.
83. Pereira R, Oliveira J, Sousa M. Bioinformatics and Computational Tools for Next-Generation Sequencing Analysis in Clinical Genetics. J Clin Med. 2020;9(1).
84. Sanders B, D'Andrea D, Collins MO, Rees E, Steward TGJ, Zhu Y, et al. Transcriptional programs regulating neuronal differentiation are disrupted in DLG2 knockout human embryonic stem cells and enriched for schizophrenia and related disorders risk variants. Nat Commun. 2022;13(1):27.
85. Khodosevich K, Sellgren CM. Neurodevelopmental disorders-high-resolution rethinking of disease modeling. Mol Psychiatry. 2023;28(1):34–43.
86. Sabariego-Navarro M, Fernández-Blanco Á, Sierra C, Dierssen M. Neurodevelopmental disorders: 2022 update. Free Neuropathol. 2022;3:8.
87. Bitta M, Kariuki SM, Abubakar A, Newton C. Burden of neurodevelopmental disorders in low and middle-income countries: A systematic review and meta-analysis. Wellcome Open Res. 2017;2:121.
88. Hamamy H, Antonarakis SE, Cavalli-Sforza LL, Temtamy S, Romeo G, Kate LP, et al. Consanguineous marriages, pearls and perils: Geneva International Consanguinity Workshop Report. Genet medicine: official J Am Coll Med Genet. 2011;13(9):841–7.
89. Naeem M, Ahmad B, Malik S. Burden of congenital and hereditary anomalies in the war-affected territory at Pakistan-Afghanistan border. Asian biomedicine: Res reviews news. 2022;16(6):299–309.
90. Iqbal S, Zakar R, Fischer F, Zakar MZ. Consanguineous marriages and their association with women's reproductive health and fertility behavior in Pakistan: secondary data analysis from Demographic and Health Surveys, 1990–2018. BMC Womens Health. 2022;22(1):118.
91. Munir A, Afsar S, Rehman AU. A systematic review of inherited retinal dystrophies in Pakistan: updates from 1999 to April 2023. BMC Ophthalmol. 2024;24(1):55.
92. Khan MS, Ahmed M, Khan RA, Mushtaq N, Wasim Ullah Shah M. Consanguinity ratio in b-thalassemia major patients in District Bannu. J Pak Med Assoc. 2015;65(11):1161–3.
93. Global burden of. 292 causes of death in 204 countries and territories and 660 subnational locations, 1990–2023: a systematic analysis for the Global Burden of Disease Study 2023. Lancet (London, England). 2025;406(10513):1811–72.
94. Riaz I, Rashid A, Majeed A. Demographic analysis of hearing impairment based on various parameters in patients with cochlear implant. J Pak Med Assoc. 2024;74(3):476–9.

95. Ahmad R, Naeem M. A systematic review of hereditary neurological disorders diagnosed by whole exome sequencing in Pakistani population: updates from 2014 to November 2024. Neurogenetics. 2025;26(1):40.
96. Farcy S, Hachour H, Bahi-Buisson N, Passemard S. Genetic Primary Microcephalies: When Centrosome Dysfunction Dictates Brain and Body Size. Cells. 2023;12(13).
97. Chang YM, Huang YT, Lai PC. Genetic testing for diagnosing neurodevelopmental disorders and epilepsy: a systematic review and meta-analysis. Syst Rev. 2025;14(1):155.
98. Alvarez-Mora MI, Rodríguez-Revenga L, Jodar M, Potrony M, Sanchez A, Badenas C et al. Implementation of Exome Sequencing in Clinical Practice for Neurological Disorders. Genes (Basel). 2023;14(4).
99. Shin S, Lee J, Kim YG, Ha C, Park JH, Kim JW, et al. Genetic Diagnosis of Children With Neurodevelopmental Disorders Using Whole Genome Sequencing. Pediatr Neurol. 2023;149:44–52.
100. Sinha S, Rabea F, Ramaswamy S, Chekroun I, El Naofal M, Jain R, et al. Long read sequencing enhances pathogenic and novel variation discovery in patients with rare diseases. Nat Commun. 2025;16(1):2500.
101. Hereditary Keratodermas In Pakistan. A Distinct Genetic Landscape Shaped By Consanguinity. Pakistan J Med Cardiological Rev. 2025;4(4):2243–54.
102. Mushtaq I, Rashid A, Fakhr A, Majeed A, Rathore A, Baig ZA. Association of single nucleotide polymorphism rs3213119 variant of IL-12B gene in diagnosed Rheumatoid Arthritis patients. Pak J Med Sci. 2024;40(5):864–9.
103. Smedley D, Smith KR, Martin A, Thomas EA, McDonagh EM, Cipriani V, et al. 100,000 Genomes Pilot on Rare-Disease Diagnosis in Health Care - Preliminary Report. N Engl J Med. 2021;385(20):1868–80.
104. Turro E, Astle WJ, Megy K, Gräf S, Greene D, Shamardina O, et al. Whole-genome sequencing of patients with rare diseases in a national health system. Nature. 2020;583(7814):96–102.
105. Khan N, Shah K, Fozia F, Khan SA, Muhammad N, Nasir A, et al. Biallelic mutations in FLG, TGM1, and STS genes segregated with different types of ichthyoses in eight families of Pakistani origin. Int J Dermatol. 2023;62(5):672–80.
106. Khan FF, Khan N, Rehman S, Ejaz A, Ali U, Erfan M et al. Identification and Computational Analysis of Novel Pathogenic Variants in Pakistani Families with Diverse Epidermolysis Bullosa Phenotypes. Biomolecules. 2021;11(5).
107. Ishaq R, Ilyas M, Habiba U, Amin MNU, Saeed S, Raja GK et al. Whole Exome Sequencing Reveals Clustering of Variants of Known Vitiligo Genes in Multiplex Consanguineous Pakistani Families. Genes (Basel). 2023;14(5).
108. Ullah MI. Clinical and Mutation Spectrum of Autosomal Recessive Non-Syndromic Oculocutaneous Albinism (nsOCA) in Pakistan: A Review. Genes (Basel). 2022;13(6).
109. Shadab M, Abbasi AA, Ejaz A, Ben-Mahmoud A, Gupta V, Kim HG, et al. Autosomal recessive non-syndromic hearing loss genes in Pakistan during the previous three decades. J Cell Mol Med.

2024;28(8):e18119.
110. Saleheen D, Natarajan P, Armean IM, Zhao W, Rasheed A, Khetarpal SA, et al. Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity. Nature. 2017;544(7649):235–9.
111. Musolf AM, Haarman AEG, Luben RN, Ong JS, Patasova K, Trapero RH, et al. Rare variant analyses across multiethnic cohorts identify novel genes for refractive error. Commun Biol. 2023;6(1):6.
112. Paracha SA, Nawaz S, Tahir Sarwar M, Shaheen A, Zaman G, Ahmed J, et al. The genetic cause of neurodevelopmental disorders in 30 consanguineous families. Front Med (Lausanne). 2024;11:1424753.
113. Bokhari N, Gillani S, Khalil GHK, Akhtar S. REPRODUCTIVE HEALTH EFFECTS OF CONSANGUINEOUS MARRIAGES IN KHYBER PAKHTUNKHWA. J Med Sci. 2015;23(1):17–20.
114. Jayaraman D, Kodani A, Gonzalez DM, Mancias JD, Mochida GH, Vagnoni C, et al. Microcephaly Proteins Wdr62 and Aspm Define a Mother Centriole Complex Regulating Centriole Biogenesis, Apical Complex, and Cell Fate. Neuron. 2016;92(4):813–28.
115. Rasool S, Baig JM, Moawia A, Ahmad I, Iqbal M, Waseem SS, et al. An update of pathogenic variants in ASPM, WDR62, CDK5RAP2, STIL, CENPJ, and CEP135 underlying autosomal recessive primary microcephaly in 32 consanguineous families from Pakistan. Mol Genet Genomic Med. 2020;8(9):e1408.
116. Naseer MI, Rasool M, Sogaty S, Chaudhary RA, Mansour HM, Chaudhary AG, et al. A novel WDR62 mutation causes primary microcephaly in a large consanguineous Saudi family. Ann Saudi Med. 2017;37(2):148–53.
117. Riazuddin S, Hussain M, Razzaq A, Iqbal Z, Shahzad M, Polla DL, et al. Exome sequencing of Pakistani consanguineous families identifies 30 novel candidate genes for recessive intellectual disability. Mol Psychiatry. 2017;22(11):1604–14.
118. Olshen AB, Venkatraman ES, Lucito R, Wigler M. Circular binary segmentation for the analysis of array-based DNA copy number data. Biostatistics. 2004;5(4):557–72.
119. Wang L, Li Z, Sievert D, Smith DEC, Mendes MI, Chen DY, et al. Loss of NARS1 impairs progenitor proliferation in cortical brain organoids and leads to microcephaly. Nat Commun. 2020;11(1):4038.
120. Efendic F, Hermann A, Frech MJ. Disrupted Myelination in FAHN: Insights from a Patient-Specific hiPSC Neuron-Oligodendrocyte Model. Cells. 2025;14(16).

Figures

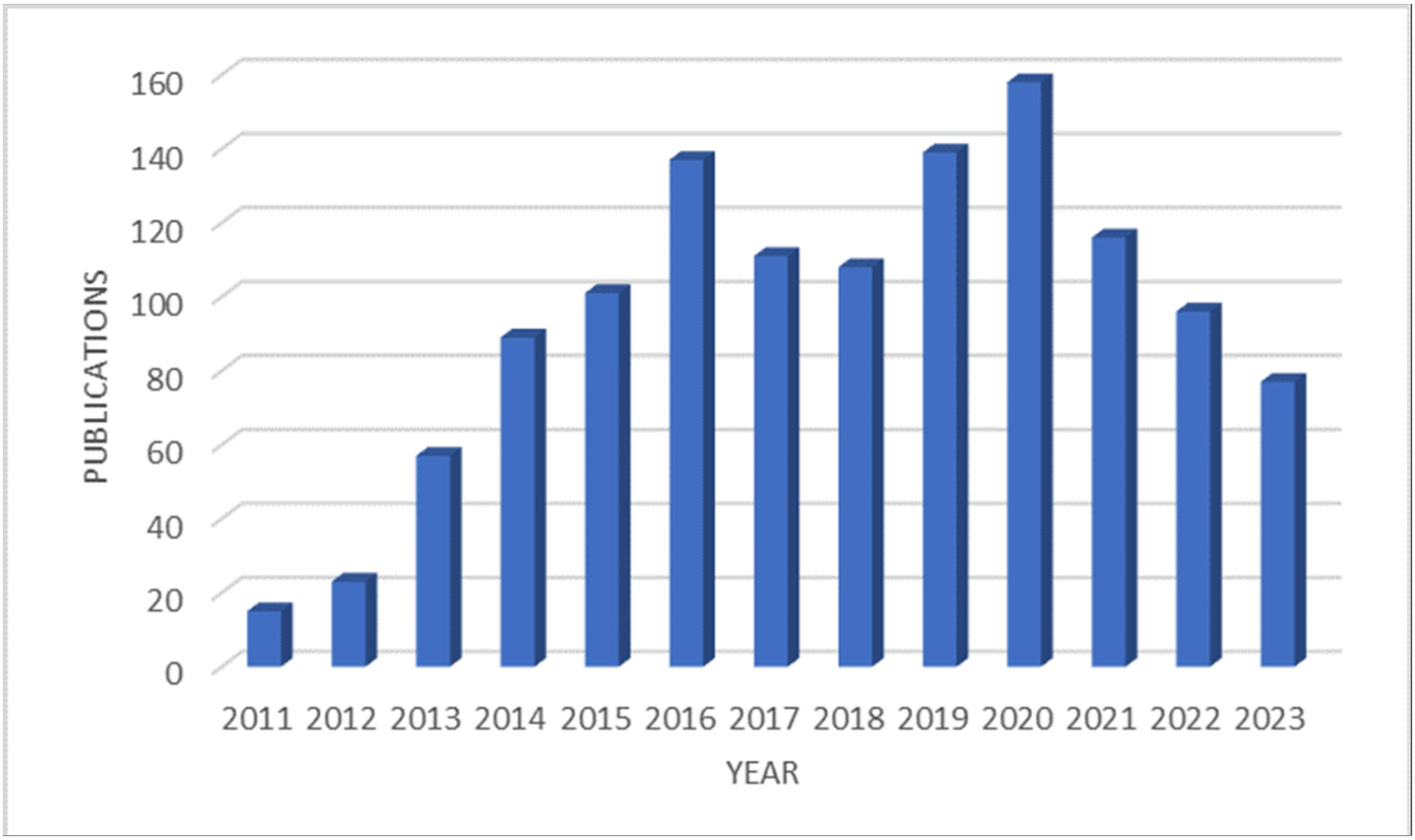


Figure 1

List of research publications from 2011 to 2023 of next generation sequencing used in intellectual disability research

a

Characteristics	N	%
1. Articles		
2006-2010	6	4%
2011-2015	27	19%
2016-2020	48	33%
2021-2024	64	44%
Total	145	
2. Parental consanguinity		
Present	458	85%
Absent	27	5%
Not sure	52	10%
Total	537	
3. Participants' health status		
Affected	815	49%
Healthy	849	51%
Total	1664	
4. Genes		
Genes found in only one family	230	80%
Genes found in multiple families	59	20%
Total	289	
5. Alleles		
Gene carrying only one allele	244	58%
Gene carrying multiple alleles	180	42%
Total	424	

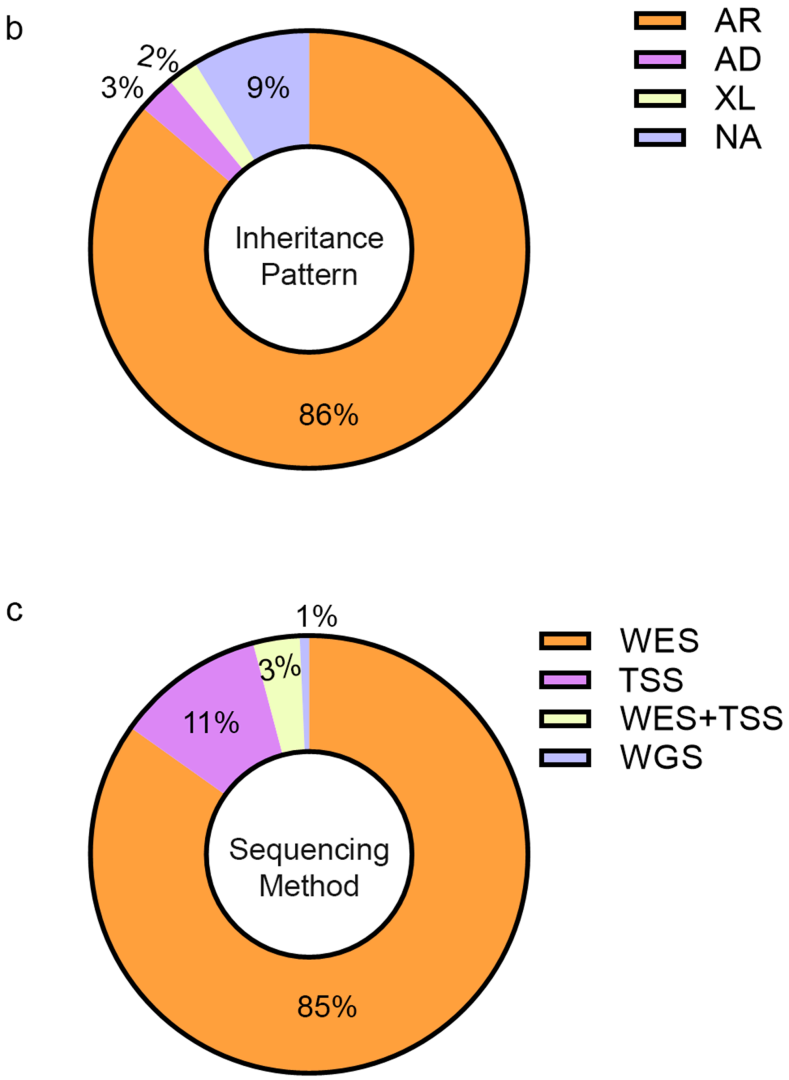


Figure 2

Overview of study characteristics, inheritance patterns, and sequencing approaches in Pakistani neurodevelopmental disorder (NDD) studies

(AR: Autosomal Recessive, AD: Autosomal Dominant, XL: X-linked, NA: Not Available; WES: Whole Exome Sequencing, TSS: Targeted Sequencing, WGS: Whole-Genome Sequencing)

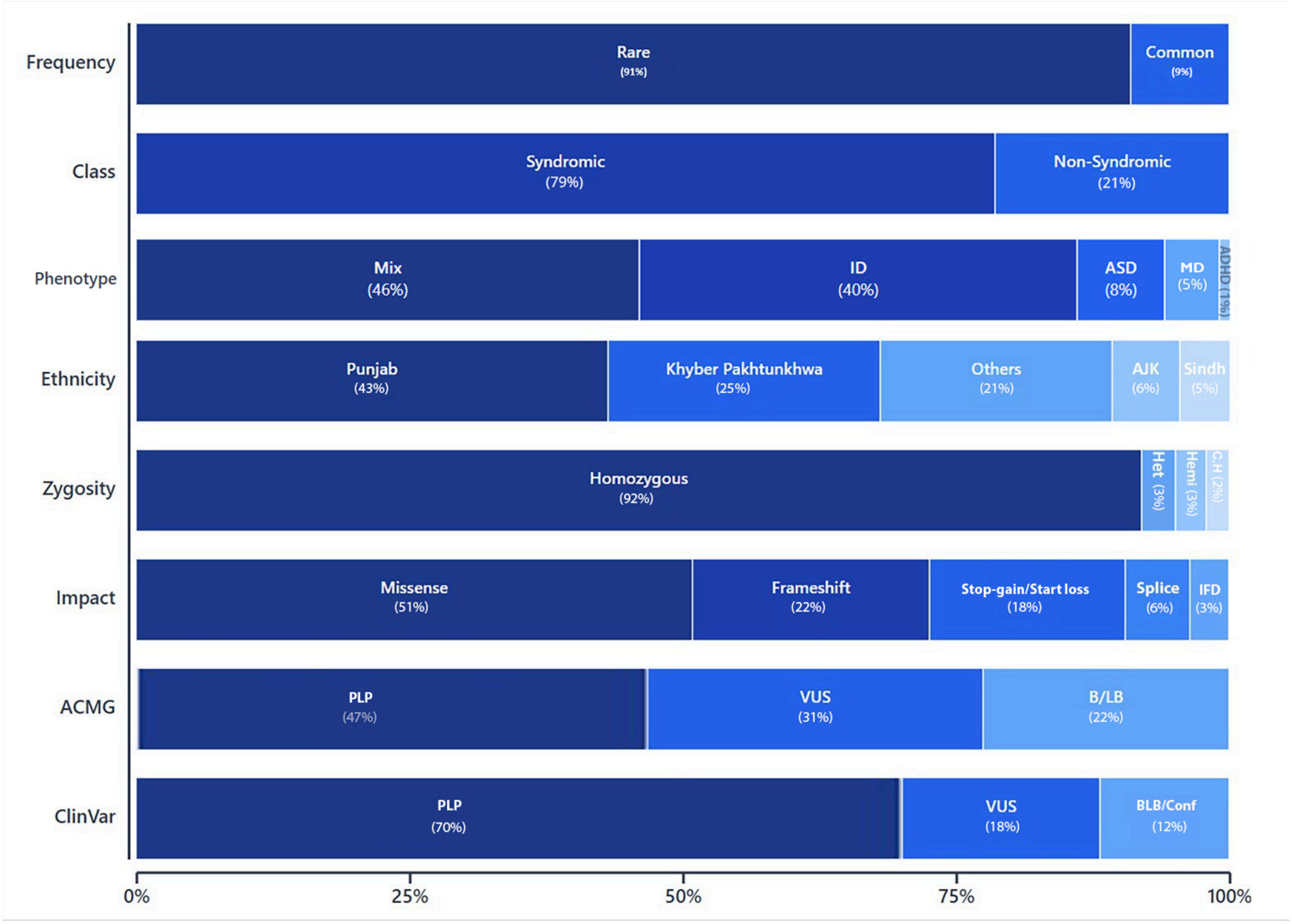


Figure 3

Genotypic and phenotypic heterogeneity distribution in NDDs of Pakistani population

(AJK: Azad Jammu Kashmir, Het: Heterozygous, C.H: Compound Heterozygous, Hemi: Hemizygous, ID: Inframe Deletions, SL: Start Loss, VUS: Variant of Uncertain Significance, B/LB: Benign/Likely Benign, B/LB/C: Benign/Likely Benign/ Conflicting)

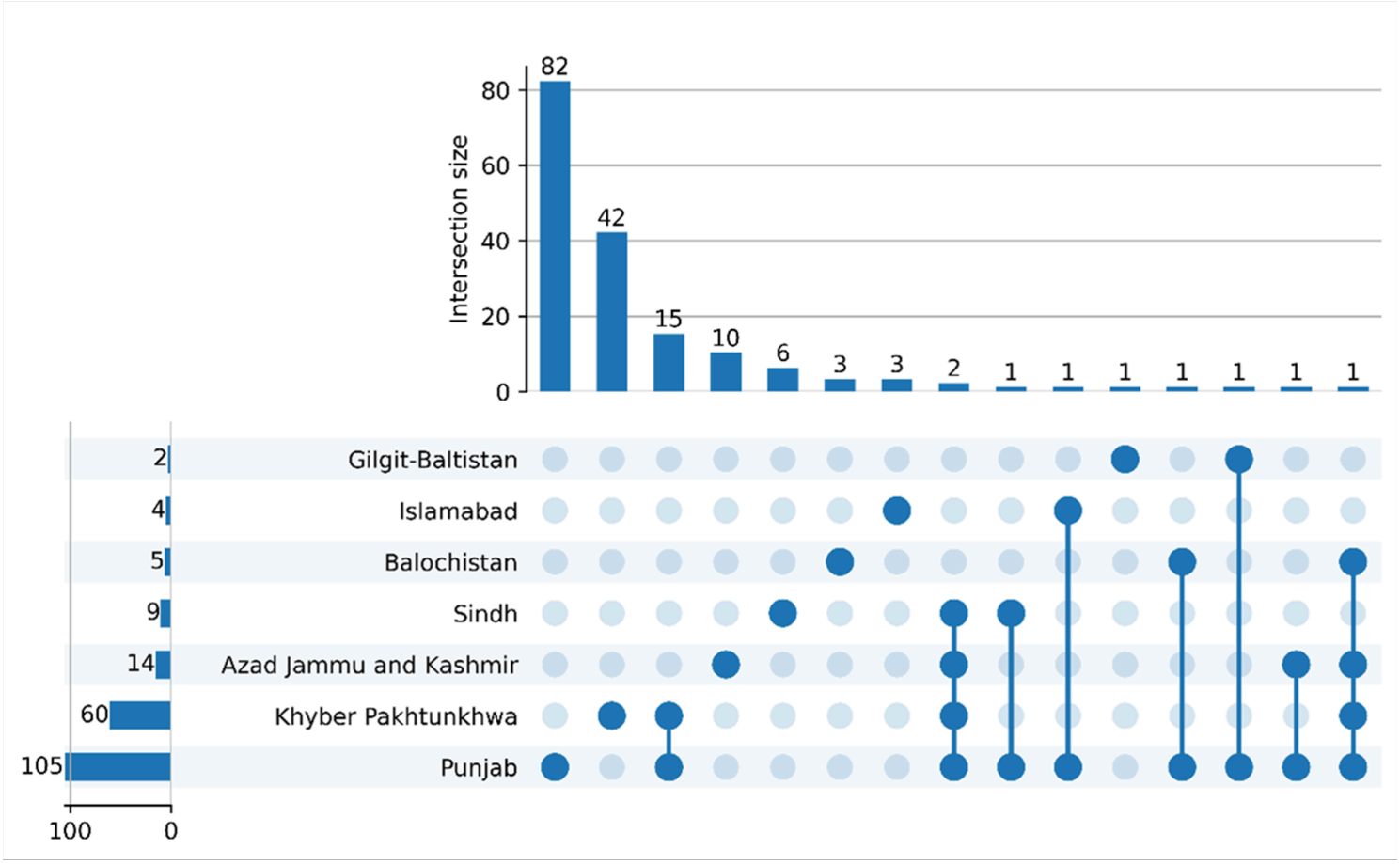


Figure 4

Gene intersections showing overlaps across seven geographic regions of Pakistan

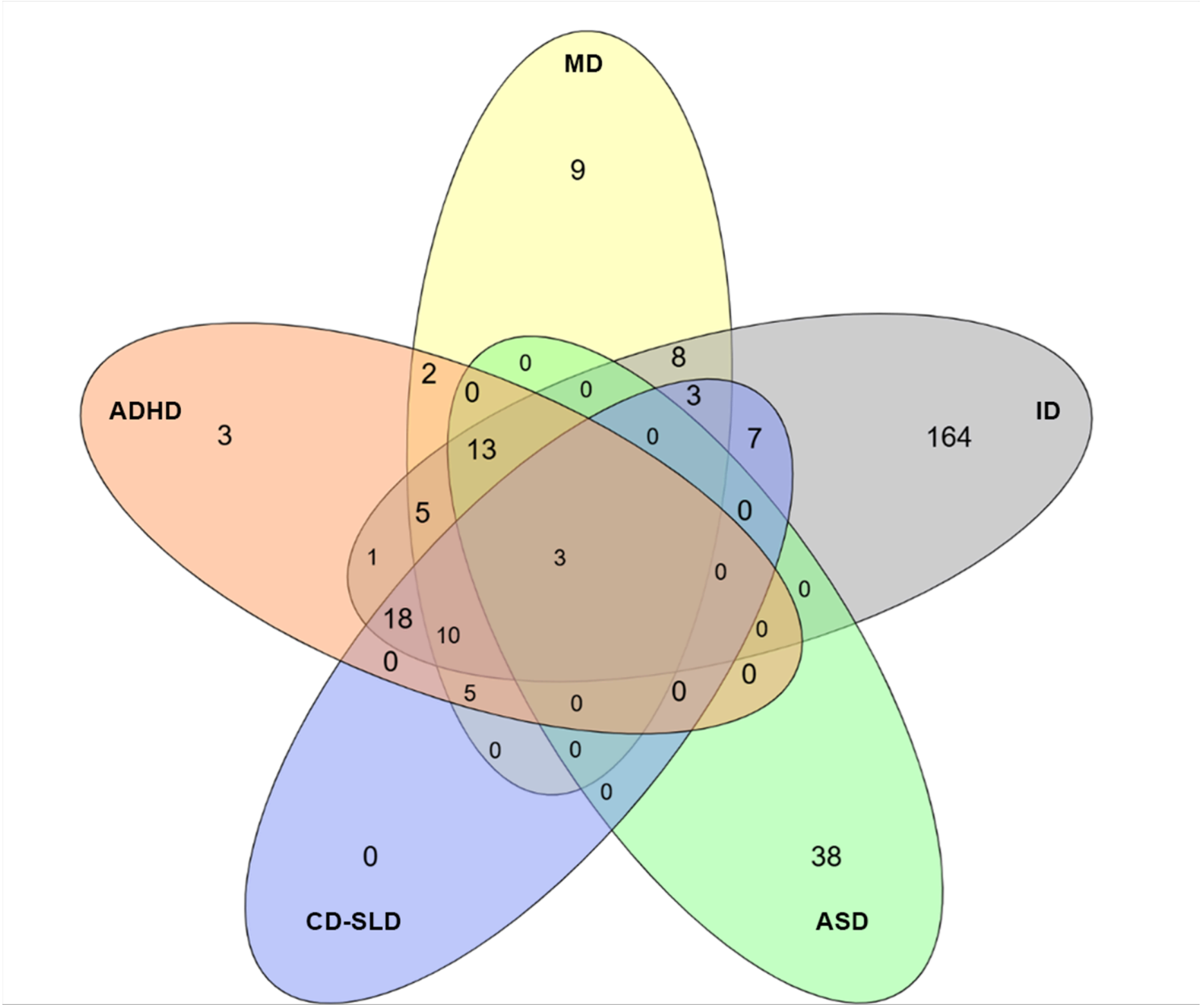


Figure 5

The shared and unique genes among Pakistani NDDs classes

(ID: Intellectual Disabilities, ADHD: Attention-Deficit/Hyperactivity Disorders, ASD: Autism Spectrum Disorders, MD: Motor Disorders, CD/SLD: Communication Disorders/Specific Learning Disorders)

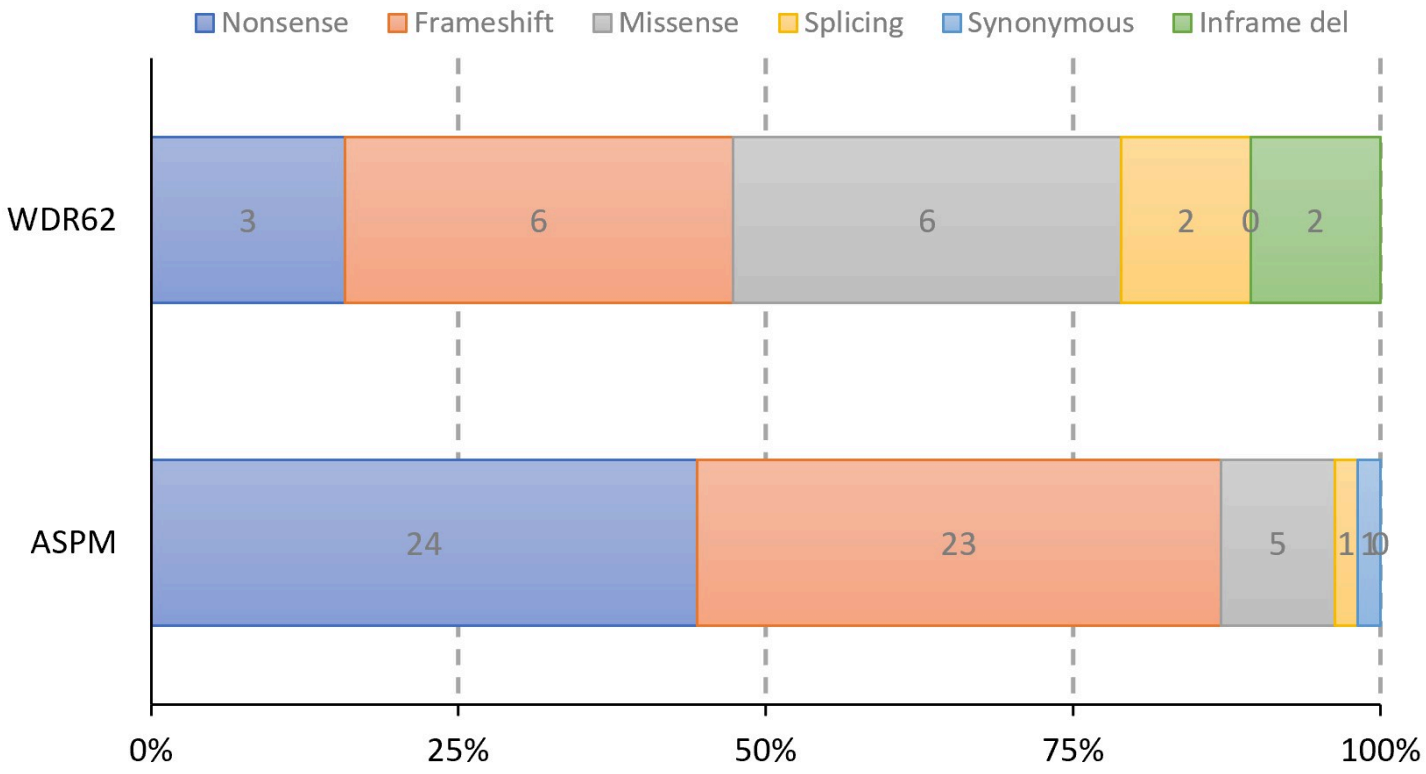


Figure 6

Bar graph showing types of variants along with their corresponding counts/numbers observed in *WDR62*and *ASPM* genes

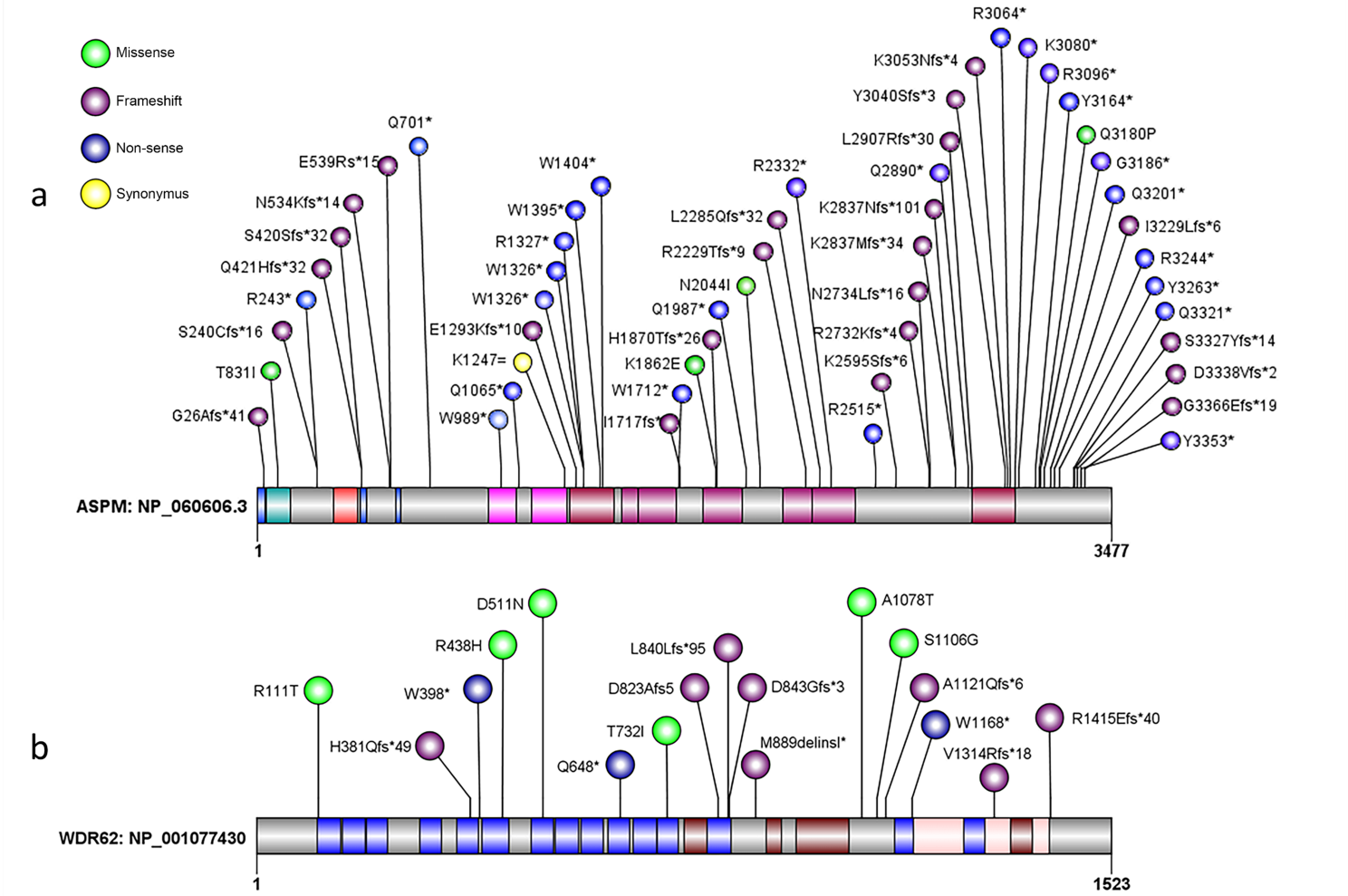


Figure 7

Spectrum of coding variants shown as vertical lollipops on the respective proteins. (a) Diagrammatic representation of coding variants reported in *ASPM*(NP_060606.3) observed in Pakistan. (b Diagrammatic representation of coding variants reported in WDR62 (NP_001077430.1) observed in Pakistan.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplFigure1.docx
- SupplTable1.xlsx
- SupplTable2.xlsx
- SupplTable3.xlsx