Introduction
1.1 Introduction

Intellectual disability (ID) is the neurodevelopmental manifestation of impaired cognitive, social and adaptive functions. Often, ID is accompanied by global developmental delay (GDD), which refers to a disturbance in an individual child across one or more developmental domains, observed as limitations or delay in the achievement of milestones and the widespread acquisition of skills, noticeable in the very early years of life [1]. As presently conceptualized, ID and GDD represent clinically defined and recognized symptom complexes that are related but not necessarily synonymous [2]. Thus, these entities share common features and at their core, both represent defects or disorders in learning [3]. Of late, the term “ID” has been replaced by the previously used “mental retardation.” According to the American Association on Intellectual and Developmental Disabilities (AAIDD), this term is preferred because it (i) better reflects the changed construct of disability, (ii) focuses on functional behaviors and contextual factors, (iii) provides a logical basis for understanding supports provision due its basis in logical framework, (iv) is less offensive to persons with disabilities, and (v) is more consistent with international terminology [4].

1.2 Definition of ID

ID is characterized by significant limitations both in intellectual functioning and in adaptive behavior as expressed in conceptual, social, and practical adaptive skills. This disability originates before the age of 18 years. The following five assumptions are essential to the application of this definition.

- Limitations in present functioning must be considered within the context of community environments typical of the individual’s age peers and culture
- Valid assessment considers cultural and linguistic diversity as well as differences in communication, sensory, motor, and behavioral factors
- Within an individual, limitations often coexist with strengths
• An important purpose of describing limitations is to develop a profile of needed supports

• With appropriate personalized supports over a sustained period, the life functioning of the person with ID generally will improve

[4] The definition of ID keeps evolving over time, as it has been proposed that in the next revision of the ICD due in 2017, it will have substituted the term “Mental Retardation” with “Intellectual Developmental Disorders”; which is described as a group of developmental conditions characterized by significant impairment of cognitive functions, which are associated with limitations of learning, adaptive behavior and skills.

1.3 Classification of ID

Historically, the primary mode of classifying persons with ID has been with the evaluation of the individual’s intelligent quotient (IQ).

1.3.1 The WHO system of classification

The system entails categorizing ID based on the IQ range of an individual. The classification is described as follows:

• Mild ID: The IQ range is 50 to 69 (in adults, mental age from 9 to under 12 years). Likely to result in some learning difficulties in school. Many adults will be able to work and maintain good social relationships and contribute to society.

• Moderate ID: The IQ ranges between 35 and 49 (in adults, mental age from 6 to under 9 years). Likely to result in marked developmental delays in childhood but most can learn to develop some degree of independence in self-care and acquire adequate communication and academic skills. Adults will need varying degrees of support to live and work in the community.

• Severe ID: Approximate IQ range of 20 to 34 (in adults, mental age from 3 to under 6 years). Likely to result in continuous need of support.
• Profound ID: IQ under 20 (in adults, mental age below 3 years). Results in severe limitation in self-care, continence, communication and mobility.

Of late, newer classification systems have emerged that consider other adaptive features along with IQ.

1.3.2 The AAIDD multidimensional approach

The AAIDD presents a classification system that uses a multidimensional approach that incorporates new knowledge and accommodates changing concepts and philosophies rather than the traditional approach of IQ range bands or adaptive behavior limitation scores. The system is based on the conceptual framework of human functioning (Figure 1.1) and incorporates IQ assessment as part of the classification, however, as one of the dimensions, rather than the sole criterion [4].

1.3.3 The American Psychological Association (APA) multiaxial system

The diagnostic and statistical manual of mental disorders, fourth edition, (DSM-IV) used a multiaxial system comprising five domains that provide information about an individual [5]. It classified “mental retardation” along with personality disorders, to
ensure that clinicians identified associated impairments alongside other mental disorders and adopting the AAIDD diagnostic criteria [6].

1.4 Prevalence

The prevalence of ID is reported to be about 1-3% in the population worldwide [7]. Some of the earliest epidemiological studies in ID are from Iceland and Denmark, where the prevalence of “mental defectives” was 2.3 and 0.9 per 1000 population, respectively [8]. Helgason [9] used the national register and estimated “intellectual subnormality” which comprised “intellectual inferiority” (IQ 75-89) and “mentally deficient” (IQ<75) groups as about 4%. The rates were found to be higher in the rural population, especially among males and those from the lowest socio-economic class. Similar excess in male child has been reported by Drillien et al [10].

In developed countries, ID represents the most frequent cause of severe handicap in children and one of the main reasons for referral in clinical genetic practices [11]. In India, ID constituted 11% of the referrals to the Genetics clinic, forming one of the top four disorders for genetic referral in the country [12]. From an epidemiologic perspective, ID prevalence rates vary according to the definitions and classification system used for evaluation (WHO 2007).

1.4.1 Worldwide prevalence estimates

Many factors affect the estimates of ID, including diagnostic criteria, severity of illness, gender, age, study population, and socioeconomic status. While most studies in the developed countries are based on IQ evaluation as well as comprehensive adaptive behavior and psychological assessments, studies from the developing countries are mostly based only on clinical assessment. Table 1.1 lists the estimated prevalence of ID in various countries.
### Table 1.1: Prevalence of ID in various countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Workup</th>
<th>Population size (n)</th>
<th>Number of ID cases</th>
<th>Prevalence/1000 population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japan</td>
<td>Psychological assessment</td>
<td>21622</td>
<td>154</td>
<td>7.12</td>
<td>[16]</td>
</tr>
<tr>
<td>Brazil</td>
<td>Clinical evaluation</td>
<td>1058</td>
<td>68</td>
<td>64.27</td>
<td>[17]</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Clinical evaluation</td>
<td>959</td>
<td>21</td>
<td>21.9</td>
<td>[17]</td>
</tr>
<tr>
<td>Philippines</td>
<td>Clinical evaluation</td>
<td>1000</td>
<td>9</td>
<td>9</td>
<td>[17]</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>Clinical evaluation</td>
<td>962</td>
<td>12</td>
<td>12.47</td>
<td>[17]</td>
</tr>
<tr>
<td>Sweden</td>
<td>Psychological &amp; Clinical evaluation</td>
<td>23544</td>
<td>170</td>
<td>7.22</td>
<td>[18]</td>
</tr>
<tr>
<td>Zambia</td>
<td>Clinical evaluation</td>
<td>1139</td>
<td>35</td>
<td>30.73</td>
<td>[17]</td>
</tr>
<tr>
<td>Denmark</td>
<td>Clinical evaluation</td>
<td>4138</td>
<td>18</td>
<td>4.35</td>
<td>[19]</td>
</tr>
<tr>
<td>Germany</td>
<td>Based on ICD</td>
<td>35026</td>
<td>245</td>
<td>6.99</td>
<td>[20]</td>
</tr>
<tr>
<td>Spain</td>
<td>Psychological assessment</td>
<td>29415</td>
<td>401</td>
<td>13.63</td>
<td>[21]</td>
</tr>
<tr>
<td>Egypt</td>
<td>Based on ICD</td>
<td>3000</td>
<td>81</td>
<td>27</td>
<td>[22]</td>
</tr>
<tr>
<td>Thailand</td>
<td>Clinical evaluation</td>
<td>4366</td>
<td>7</td>
<td>1.6</td>
<td>[23]</td>
</tr>
<tr>
<td>Norway</td>
<td>Based on DSM</td>
<td>30037</td>
<td>185</td>
<td>6.16</td>
<td>[24]</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Psychological assessment</td>
<td>423000</td>
<td>11892</td>
<td>28.11</td>
<td>[25]</td>
</tr>
<tr>
<td>USA</td>
<td>Clinical evaluation</td>
<td>35704</td>
<td>1312</td>
<td>36.75</td>
<td>[26]</td>
</tr>
<tr>
<td>Canada</td>
<td>Based on ICD</td>
<td>35485</td>
<td>255</td>
<td>7.19</td>
<td>[27]</td>
</tr>
<tr>
<td>South Africa</td>
<td>Psychological assessment</td>
<td>6692</td>
<td>238</td>
<td>35.56</td>
<td>[28]</td>
</tr>
</tbody>
</table>

*continued*
<table>
<thead>
<tr>
<th>Country</th>
<th>Workup</th>
<th>Population size (n)</th>
<th>Number of ID cases</th>
<th>Prevalence/1000 population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saudi Arabia</td>
<td>Psychological assessment</td>
<td>60630</td>
<td>540</td>
<td>8.9</td>
<td>[29]</td>
</tr>
<tr>
<td>Finland</td>
<td>Based on ICD</td>
<td>5184980</td>
<td>36053</td>
<td>6.95</td>
<td>[30]</td>
</tr>
<tr>
<td>India</td>
<td>Psychological assessment</td>
<td>550000</td>
<td>511</td>
<td>0.93</td>
<td>[31]</td>
</tr>
<tr>
<td>Pakistan</td>
<td>Psychological &amp; Clinical evaluation</td>
<td>1476</td>
<td>54</td>
<td>36.59</td>
<td>[32]</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>Based on ICF</td>
<td>24453</td>
<td>97</td>
<td>3.97</td>
<td>[33]</td>
</tr>
<tr>
<td>Ireland</td>
<td>Based on ICD</td>
<td>2776587</td>
<td>16794</td>
<td>6.05</td>
<td>[34]</td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>Based on ICD</td>
<td>1185114</td>
<td>8340</td>
<td>7.04</td>
<td>[34]</td>
</tr>
<tr>
<td>Australia</td>
<td>Based on AAIDD</td>
<td>474285</td>
<td>6106</td>
<td>12.87</td>
<td>[35]</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>Psychological &amp; Clinical evaluation</td>
<td>766</td>
<td>2</td>
<td>2.61</td>
<td>[36]</td>
</tr>
<tr>
<td>Cuba</td>
<td>Psychological &amp; Clinical evaluation</td>
<td>183871</td>
<td>1140</td>
<td>6.2</td>
<td>[15]</td>
</tr>
<tr>
<td>China</td>
<td>Based on ICF</td>
<td>60124</td>
<td>560</td>
<td>9.31</td>
<td>[37]</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Based on AAIDD</td>
<td>1142679</td>
<td>7352</td>
<td>6.43</td>
<td>[38]</td>
</tr>
</tbody>
</table>

*Source: [15]*
1.4.2 Indian scenario

According to the census of India, 2001 [13], 2.13% of the population was reported to have mental disability, which is about 2.1 million people of the 21 million people with disabilities. However, in 2011, when the new category of “mental retardation” was added, the number of individuals with the disability was estimated to be 1.5 million, of which, 0.8 million were males and 0.6 million were females [14].

1.5 Etiology

The etiology of ID is diverse as it is a complex, multifactorial disorder. The contribution of various etiological factors to the incidence of ID is presented in Figure 1.2.

![Figure 1.2: Etiology of Intellectual Disability](image)

1.5.1 Environmental/teratogenic factors

Various environmental factors may result in ID and GDD in the developing child. These include exposures to heavy metals, toxins, pathogens, and malnutrition, both in the prenatal and postnatal phases.
1.5.1.1 Exposure to toxins

During prenatal neurodevelopment, and in the first years of life, the brain is particularly vulnerable to the neurotoxic effects of exposures to toxins. In specific, neurotoxicants interfere with essential developmental central nervous system processes [39]. Among many toxicants, lead is a heavy metal that damages the nervous system, leading to decreased learning ability and behavioral deficits [40]. Similarly, mercury is a known teratogen and prenatal exposures to mercury can impair many neurocognitive functions, including thought processing, memory, attention, and fine motor skills. Both metals have the ability to cross the placenta from the maternal circulation into the fetus [41]. Children born to mothers who smoked during their pregnancy have higher rates of learning disorders, attention deficit/hyperactivity disorders, and disruptive behaviors [42]. Prenatal exposure to ionizing radiation is also a well-known risk factor for ID [43]. Thus, the exposure to various environmental agents at specific periods during development contribute significantly to ID.

1.5.1.2 Exposure to pathogens

Childhood diseases that result from a prenatal/postnatal infection such as TORCH (Toxoplasmosis; Other infections including whooping cough, chicken pox, measles, and Hib disease; Rubella; Cytomegalovirus infections; and Herpes simplex II infections) infections may lead to meningitis and encephalitis, which can damage the brain and in turn contribute to a defect in the brain function including ID [44].

1.5.1.3 Malnutrition

Maternal malnutrition, in particular, lack of essential micronutrients, can affect the development of the fetus, cause intra-uterine growth delay and increase the risk of impaired development of the infant. It is well documented that low maternal folate is associated with an increased risk of neural tube defects [45]. Deficiency of vitamin D and/or calcium during pregnancy is a risk factor for pre-term birth, which is associated
with numerous complications and adverse long-term sequelae including cerebral palsy and cognitive, visual and hearing impairments [46]. Iodine deficiency, especially during early pregnancy, is a major global cause of impaired cognitive development [47]. Iron deficiency is also common and can affect fetal brain structure and function, leading to cognitive and behavioral impairments [48].

1.5.1.4 Fetal alcohol syndrome

Maternal substance abuse, including alcohol, is known to increase the risk of developmental defects in the fetus. Fetal alcohol syndrome (FAS) is characterized by mild to moderate ID, decreased growth in utero, microcephaly, hypotonia, and facial dysmorphism with an incidence of 1.3-4.8 per 1000 live births [49]. Development of facial features of FAS is dependent upon the time of pregnancy and fetal exposure to alcohol [50]. The neurological damage caused by alcohol exposure includes loss of brain cells, structural brain malformations such as microcephaly, abnormal shape and size of corpus callosum and cerebral hypoplasia [51–53].

1.5.2 Complications of prematurity

It is reported that the risk of ID/GDD increases exponentially with decreasing gestational age [54]. There is a rapid growth of the central nervous system during the third trimester. Between 24 and 40 weeks of gestation, the fetus’s cortical volume increases fourfold and there is a dramatic increase in the number of neurons, axons and synapses, increased myelination and more complex brain activity. Although maturation continues after delivery, the conditions for optimal development occur in utero [55]. Children born before 36 weeks of gestation are at risk for deficits in intellectual and adaptive functioning in infancy and early childhood. The problems of prematurity include serious health conditions such as cerebral palsy, chronic lung disease, vision and hearing loss, intracranial hemorrhage and hydrocephalus [56]. Complications that occur during gestation, in addition to increasing the risk of a preterm birth, can also contribute to ID.
Maternal hypertension or uncontrolled diabetes during pregnancy are occasionally associated with ID in children. Perinatal complications include birth asphyxia or hypoxic ischemic encephalopathy [44].

### 1.5.3 Cultural/familial factors

Cultural/familial ID is said to result from the interaction of genetic and environmental factors over time, where there is a genetic propensity towards low intelligence complicated by environmental deprivations that prevent the wholesome development of a child’s cognitive abilities. Environmental deprivation might include poor access to health care, inadequate nutrition, lack of cognitive stimulation during early childhood, low-quality educational experiences and lack of cultural experiences [44]. It is reported that children from low-income families or socioeconomic status often earn lower IQ scores [57]. Familial ID is also more frequent among ethnic minorities; compounded by the fact that ethnic minorities often belong to the lower socioeconomic stratum [58].

### 1.5.4 Structural central nervous system abnormalities

A number of structural central nervous system (CNS) abnormalities have been associated with ID, including neural tube defects (NTD), holoprosencephaly, microcephaly, macrocephaly, lissencephaly, to name a few. Neural tube defects occur in approximately 1 per 1000 pregnancies [59]. These defects arise due to a fault in the neurulation process that occurs in the third week of gestation. Spina bifida (Menigocele and myelomeningocele) is a NTD with a risk for hydrocephalus, which could result in ID [60–62]. Holoprosencephaly occurs due to improper cleavage of the embryonic forebrain, its expression varies from mild to severe, along with varying degrees of facial dysmorphism. It may be caused due to genetic as well as environmental factors [63]. Lissencephaly is caused by defective neuronal migration during the 12th to 24th weeks of gestation, resulting in a lack of development of gyri and sulci and similar to holoprosencephaly, both genetic as well as environmental factors have been implicated [64].
1.5.5 Metabolic and endocrine factors

Inborn errors of metabolism are congenital deficiencies of key metabolic enzymes. Major categories of disorders include aminoacidopathies, organic acidemias, lysosomal storage disorders, urea cycle defects, primary lactic acidosis, fatty acid oxidation defects, disorders of carbohydrate metabolism and peroxisomal disorders.

1.5.5.1 Phenylketonuria

Phenylketonuria is an aminoacidopathy caused by mutations of the phenylalanine hydroxylase (PAH) gene or of the genes coding for enzymes involved in the cofactor tetrahydrobiopterin biosynthesis or recycling, which result in dysfunction of phenylalanine metabolism leading to excessive phenylalanine and related substances in the blood, brain and urine. Increased phenylalanine concentrations in the brain are toxic and cause disruption of neuropsychological function. The prevalence varies widely, and is 1 in 10000 live births in Europe [65, 66].

1.5.5.2 Congenital hypothyroidism

Hypothyroidism is an endocrine disorder caused by thyroid hormone deficiency. It can be classified into either permanent or transient; the former is usually associated with thyroid dysgenesis, dysfunction of thyroid hormone biosynthesis or metabolism or deficiency of thyroid stimulating hormone which may occur due to iodine deficiency, maternal intake of antithyroid drugs or transplacental maternal thyrotropin receptor blocking antibodies (TRB-Ab). If untreated, congenital hypothyroidism can result in growth failure, permanent intellectual impairment and cardiac problems. Congenital hypothyroidism occurs in approximately 1 per 2000 to 4000 live births [67].
1.5.6 Chromosomal abnormalities

Chromosome abnormalities are microscopically visible alterations of chromosomes, caused by errors in either mitosis or meiosis. They can result in either numerical abnormalities such as aneuploidies or structural anomalies comprising deletions, duplications, inversions and translocations of both, autosomes and sex chromosomes [68]. Dave et al [69] reported a frequency of 31.5% chromosomal abnormalities in the study of children with ID over 14 years, which was significantly higher than other reports (15%–28%), emphasizing the need for cytogenetic evaluation in all children with ID with or without dysmorphic features.

Down syndrome or trisomy 21 arises mostly from maternal nondisjunction, resulting in three copies of chromosome 21. Apart from free trisomy 21, robertsonian translocations of chromosome 21 or mosaic conditions have also known to result in Down syndrome. It is the leading genetic cause of ID in children and occurs in 1 in 1,000 to 1,100 live births worldwide [70]. Affected individuals have characteristic facial appearance, ID/GDD, and a risk to develop several medical conditions. Other aneuploidies include Edward syndrome (trisomy 18), Patau syndrome (trisomy 13), Turner syndrome (monosomy X) and Klinefelter syndrome (XXY).

Structural abnormalities such as inversions and balanced translocations are mostly not pathogenic in the individuals carrying them as they do not result in genomic imbalance. However, deletions and duplications result in a disease phenotype due to the loss or gain of genetic material, respectively. Structural abnormalities that involve entire chromosome arms such as Wolf-Hirschhorn syndrome (4p deletion), Cri-du-chat syndrome (5p deletion) and de Grouchy syndrome (18p deletion) are well-recognized. They are relatively rare with incidence rates ranging between 1 in 20000 and 1 in 50000 [71–73].
1.5.7 Recognized syndromes

Research in ID over the years has led to the characterization and documentation of numerous syndromes. They may be caused by monogenic or contiguous gene defects. Of the many associated syndromes, a few well-described disorders are listed below.

1.5.7.1 Fragile X syndrome

Fragile X syndrome (FXS) is the leading cause of inherited ID [74] and is the second most common cause for ID after Down syndrome. While it affects about 1 in 5000 males [75] and 1 in 4000-6000 females [76], approximately 1 in 151 females and 1 in 468 males carry the premutation [76]; i.e., females predominantly remain carriers. FXS results from the loss of function of the gene, FMR1 on chromosome Xq27.3 (FRAXA1). Clinical features of the condition include a moderate ID, seizures, long narrow face, large protruding ears and macroorchidism. Some children exhibit autistic behaviors and attention deficit [77, 78]. Oberle et al [79] and Yu et al [80] reported that a 550 bp segment of repetitive DNA (CGG trinucleotide repeats) in the FRAXA1 region is subject to size changes, and its expansion is associated with increased methylation of the CGG repeat. In normal individuals, the number of CGG repeats ranges from 6 to 55. In the case of premutation, the number of repeats increases to somewhere between 55 and 200. In the female carriers, during meiosis, the premutation may expand to a full mutation, ranging between 200 and 1000 repeats, resulting in FXS in the male offspring [81]. Other than the repeat expansion, deletions and point mutations have also been reported [82]. FRAXE is a fragile site on Xq28, in which repeat expansions result in a milder ID phenotype [83].

1.5.7.2 Williams-Beuren syndrome

Williams-Beuren syndrome (WBS) is caused by the haploinsufficiency of genes in the 7q11.23 region due to a deletion of about 1.5 Mb in the region [84]. Clinical features include moderate ID, mild growth retardation, heart defects, wide mouth, full cheeks and
lips and stellate iris pattern. The deletion is caused due to misalignment and unequal crossing over enabled by three low copy repeats flanking the region [85]. The ELN gene present in the region is involved in the pathogenesis of supravalvular aortic stenosis [86]. Other genes that also play a key role in the pathophysiology of WBS include \textit{LIMK1}, \textit{CLIP2 (CYLN2)} [87]. The prevalence of WBS is estimated to be 1 in 10000 [88].

1.5.7.3 Prader-Willi syndrome

The absence of the products of genes located in the 15q11-q13 region (which is subject to genomic imprinting) of the paternally derived chromosome 15 give rise to Prader-Willi syndrome (PWS). PWS affects approximately 1 in 15000 to 1 in 30000 individuals [89]. Clinical features of this condition include ID, GDD, hypotonia and feeding and behavioral problems. Although in about 70% of PWS cases, the cause is a deletion in the 15q11-q13 region, around 15% is due to maternal uniparental disomy, and in 2.5% of affected children, it is due to mutations in the PW imprint control region that may include the \textit{SNRPN} gene promoter and its first exon [90]. The roles of one or other genes such as \textit{NIPA1, NIPA2, CYFIP1} and \textit{TUBGCP}, located in this region, are also implicated in the pathogenesis. Angelman syndrome is a condition that arises from genetic abnormalities in the same region, however, in the maternally derived chromosome 15 or due to paternal uniparental disomy [91]. The clinical features and the genes implicated are markedly different between the two syndromes.

1.5.8 Monogenic disorders

While it was thought that ID was the side effect of a generalized metabolic disorder, Turner [92] observed in some families that genes, when abnormal, caused nonsyndromic ID. Further evidence that single genes may contribute to ID came from the reports of a number of families where ID was unusually common and was transmitted in a Mendelian fashion. In many cases, the trait was inherited as an X-linked recessive condition, where males were more frequently affected than females. Subsequently, FXS was described and the \textit{FMRI} gene, identified much later [79, 93, 94]. Currently, it is estimated that
more than 70 (10% of genes on chromosome X) genes on the X chromosome, when mutated, give rise to a syndrome with associated ID or a nonsyndromic ID. Since 2000, the rate of identification of novel genes that cause ID has progressed rapidly due to technological developments. The Online Mendelian Inheritance in Man (OMIM) lists more than 1000 entries for ID, which is an indication to the heterogeneity of the disorder. A few genes involved in the etiology of ID are listed in

1.5.9 Unknown etiology/idiopathic ID

The cause of ID remains unexplained in about 30%-50% of the affected individuals and it is reasonable to suppose that a considerable proportion of cases of unknown etiology may have a genetic origin [95]. A number of studies in the recent years have shown chromosomal rearrangements to be an important cause, resulting in segmental aneuploidy and gene dosage alteration of developmental genes [96–99]. With the advent of newer genetic techniques, several new cryptic chromosomal aberrations have been discovered in the last few years; as a result, a consistent number of ID cases, previously considered “idiopathic”, are now classified as syndromic conditions with clinically recognizable phenotypes [100].

In order to identify the cause of ID, except in cases of X-linked ID wherein the pattern of inheritance implies that the causative link can be confined to the X-chromosome, one may wish to examine the entire genome at high resolution. Such an approach, though possible at present, is neither ideal for a large cohort nor cost-effective. However, a compromise is to analyze subsegments of the genome that contain a relatively higher density of genes and have a relatively higher probability of rearrangement [95]. It has been suggested that the chromosome telomeres may represent such regions [97, 101]. The ends of all eukaryotic chromosomes are composed of a TG-rich repeat, (TTAGGG)n, ranging from 2 to 15 kb in length [102, 103]. Without the terminal array, chromosomes are unstable, and prone to end to end fusions and exonucleolytic degradation. Immediately adjacent to the (TTAGGG)n tandem repeats lie complex families of repetitive
### Table 1.2: A few monogenic causes of ID

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genes involved in neurogenesis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCPH1</td>
<td>8p22</td>
<td>Cell cycle control and DNA repair</td>
<td>Microcephaly vera</td>
</tr>
<tr>
<td>CDK5RAP2</td>
<td>9q33.2</td>
<td>Mitotic spindle function in embryonic neuroblasts</td>
<td>Microcephaly vera</td>
</tr>
<tr>
<td>ASPM</td>
<td>1q31</td>
<td>Mitotic spindle formation during mitosis and meiosis</td>
<td>Microcephaly vera</td>
</tr>
<tr>
<td>CENPJ</td>
<td>13q12.2</td>
<td>Localization to the mitotic spindle poles of mitotic cells</td>
<td>Microcephaly vera</td>
</tr>
<tr>
<td><strong>Genes involved in neuronal migration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIS1</td>
<td>17p13.3</td>
<td>Interacts with dynein and has multiple functions including role in nuclear migration and differentiation</td>
<td>Miller Dieker syndrome: type 1 lissencephaly, pachygyria, subcortical band heterotopia (double cortex)</td>
</tr>
<tr>
<td>DCX/Dbcn</td>
<td>Xq22.3</td>
<td>Microtubule-associated protein (MAP)</td>
<td>type 1 lissencephaly, pachygyria, subcortical band heterotopia (double cortex)</td>
</tr>
<tr>
<td>RELN</td>
<td>7q22</td>
<td>Extracellular matrix (ECM) molecule, reelin pathway</td>
<td>Lissencephaly with cerebellar hypoplasia</td>
</tr>
<tr>
<td>VLDLR</td>
<td>9p24</td>
<td>Low-density lipoprotein receptor, reelin pathway</td>
<td>Lissencephaly with cerebellar hypoplasia</td>
</tr>
<tr>
<td>POMT1</td>
<td>9q34</td>
<td>Protein α-mannosyl-transferase 1 (glycosylation of α-dystroglycan)</td>
<td>Walker-Warburg (HARD) syndrome</td>
</tr>
</tbody>
</table>

*continued*
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>POMT2</td>
<td>14q24.3</td>
<td>Protein o-mannosyl-transferase 2 (glycosylation of α-dystroglycan)</td>
<td>Walker-Warburg syndrome</td>
</tr>
<tr>
<td>POMGnT1</td>
<td>1p34</td>
<td>Protein o-mannose-n-acetyl-glucosaminyl-transferase</td>
<td>Muscle-eye-brain disease</td>
</tr>
<tr>
<td>FKTN (Fukutin)</td>
<td>9q31</td>
<td>Homology with glycoprotein-modifying enzyme</td>
<td>Fukuyama congenital muscular dystrophy (FCMD) with type 2 lissencephaly</td>
</tr>
<tr>
<td>FLNA</td>
<td>Xq28</td>
<td>Filamin-1 (actin crosslinking phosphoprotein)</td>
<td>Bilateral periventricular nodular heterotopia (BPNH)</td>
</tr>
</tbody>
</table>

**Genes with a role in cellular processes involved in neuronal and synaptic functions**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>FID1</td>
<td>Xq27</td>
<td>m-RNA-binding protein, role in translation, regulation by RhoGTPase pathways, postsynaptic localization</td>
<td>Fragile X syndrome</td>
</tr>
<tr>
<td>FGD1</td>
<td>Xp11.2</td>
<td>RhoGEF protein (GTP exchange factor), activate Rac1 and Cdc42</td>
<td>Aarskog-Scott syndrome</td>
</tr>
<tr>
<td>PAK3</td>
<td>Xq21.3</td>
<td>P21-activated kinase 3; effector of Rac1 and Cdc42</td>
<td>Nonsyndromic XLID</td>
</tr>
<tr>
<td>ARHGEF6</td>
<td>Xq26</td>
<td>RhoGEF protein, integrin-mediated activation of Rac1 and Cdc42</td>
<td>Nonsyndromic XLID</td>
</tr>
<tr>
<td>OPHN1</td>
<td>Xq12</td>
<td>RhoGAP protein (negative control of RhoGTPases; stimulates GTPase activity of RhoA, Rac1 and Cdc42; pre- andpostsynaptic localization)</td>
<td>ID with cerebellar hypoplasia</td>
</tr>
</tbody>
</table>

*continued*
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM4SF2</td>
<td>Xq11</td>
<td>Member of the tetraspanin family, integrin mediated RhoGTPase pathway regulation</td>
<td>Nonsyndromic XLID</td>
</tr>
<tr>
<td>NLGN4</td>
<td>Xp22.3</td>
<td>Member of the neuroligin family, role in synapse formation and activity; post synaptic localization</td>
<td>Nonsyndromic XLID, autism, Asperger syndrome</td>
</tr>
<tr>
<td>DLG3</td>
<td>Xq13.1</td>
<td>Protein involved in postsynaptic density structures; postsynaptic localization</td>
<td>Nonsyndromic XLID</td>
</tr>
<tr>
<td>GDI1</td>
<td>Xq28</td>
<td>Regulation of Rab4 and Rab5 activity, and of synaptic vesicle recycling; pre- and postsynaptic localization</td>
<td>Nonsyndromic XLID</td>
</tr>
<tr>
<td>IL1RAPL</td>
<td>Xp22.1</td>
<td>Potential involvement in exocytosis and ion channel activity</td>
<td>Nonsyndromic XLID</td>
</tr>
</tbody>
</table>

**Transcription signaling cascade, remodeling and transcription factors**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>17q11</td>
<td>RasGAP function, involved in Ras/ERK/MAPK signaling transcription cascade; postsynaptic protein</td>
<td>Neurofibromatosis type 1 (NF1); ID present in 50% NF1 individuals</td>
</tr>
<tr>
<td>RSK2</td>
<td>Xp22.2</td>
<td>Serine-threonine protein kinase, phosphorylates CREB, involved in Ras/ERK/MAPK signaling cascade, present in the postsynaptic compartment</td>
<td>Coffin-Lowry syndrome (facial and skeletal anomalies)</td>
</tr>
<tr>
<td>CDKL5</td>
<td>Xp22.2</td>
<td>Serine-threonine kinase (STK9), interacts with MECP2, potential implication in chromatin remodeling</td>
<td>Rett-like syndrome with infantile spasms</td>
</tr>
</tbody>
</table>

*continued*
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREBBP</td>
<td>16p13.3</td>
<td>CREB (cAMP response element-binding protein 1) binding protein; chromatin remodeling factor involved in Ras/ERK/MAPK signaling cascade</td>
<td>Rubinstein–Taybi syndrome</td>
</tr>
<tr>
<td>EP300</td>
<td>22q13.1</td>
<td>Transcriptional coactivator similar to CREBBP, with potent histone acetyl transferase; chromatin-remodeling factor</td>
<td>Rubinstein–Taybi syndrome</td>
</tr>
<tr>
<td>XNP</td>
<td>Xq13</td>
<td>Homology with DNA helicases of the SNF2/SWI2 family, chromatin-remodeling factor, regulation of gene expression</td>
<td>Large spectrum of phenotypes including ATRX syndrome</td>
</tr>
<tr>
<td>MECP2</td>
<td>Xq28</td>
<td>Methyl-CpG-binding protein 2; chromatin remodeling factor, involved in a transcriptional silencer complex</td>
<td>Rett syndrome (female-specific syndrome) and nonsyndromic ID</td>
</tr>
<tr>
<td>DNMT3B</td>
<td>20q11.2</td>
<td>DNA methyl transferase 3B, involved in chromatin remodeling</td>
<td>ICF syndrome (immune deficiency associated with centromeric instability, facial dysmorphology and ID)</td>
</tr>
<tr>
<td>ARX</td>
<td>Xp22.1</td>
<td>Transcription factor of the aristaless homeoprotein-related proteins family</td>
<td>Large spectrum of ID phenotypes: XLAG (X-linked lissencephaly and abnormal genitalia); West syndrome, Partington syndrome; nonsyndromic ID</td>
</tr>
<tr>
<td>JARID1C</td>
<td>Xp11.2</td>
<td>Transcription factor and chromatin remodeling</td>
<td>Spectrum of phenotypes: ID with microcephaly, short stature, epilepsy, facial anomalies and nonsyndromic ID</td>
</tr>
</tbody>
</table>
| FID2   | Xq28   | Potential transcription factor                                                               | Nonsyndromic ID                                                          

_table continued_
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX3</td>
<td>Xq27</td>
<td>SRY-BOX 3: transcription factor</td>
<td>Isolated GH deficiency, short stature and ID</td>
</tr>
<tr>
<td>PHF8</td>
<td>Xp11.2</td>
<td>PHD zinc-finger protein, potential role in transcription</td>
<td>ID with cleft lip or palate</td>
</tr>
<tr>
<td>ZNF41</td>
<td>Xp11.2</td>
<td>Potential transcription factor</td>
<td>Nonsyndromic ID</td>
</tr>
<tr>
<td>GTF2I/GTF2RD1</td>
<td>7q11.23</td>
<td>Transcription factors, potential regulator of c-Fos and immediate-early gene expression</td>
<td>WBS</td>
</tr>
<tr>
<td>PHF6</td>
<td>Xq26</td>
<td>Homeodomain-like transcription factor</td>
<td>Börjeson–Forssman–Lehmann syndrome</td>
</tr>
</tbody>
</table>

Other genes involved in ID

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPSS12</td>
<td>4q24</td>
<td>Member of the trypsin-like serine protease family, enriched in the presynaptic compartment</td>
<td>Nonsyndromic autosomal recessive (AR) ID</td>
</tr>
<tr>
<td>CRBN</td>
<td>3p25</td>
<td>ATP-dependent protease; regulation of mitochondrial energy metabolism</td>
<td>Nonsyndromic ARID</td>
</tr>
<tr>
<td>CC2D1A</td>
<td>19p13</td>
<td>Unknown function, protein contains C2 and DM14 domains</td>
<td>Nonsyndromic ARID</td>
</tr>
<tr>
<td>FTSJ1</td>
<td>Xq11.2</td>
<td>Role in tRNA modification and IDNA translation</td>
<td>Nonsyndromic XLID</td>
</tr>
<tr>
<td>PQBP1</td>
<td>Xq11.2</td>
<td>Polyglutamine-binding protein, potentially involved in pre-mRNA splicing</td>
<td>Large spectrum of ID phenotypes including nonsyndromic ID</td>
</tr>
<tr>
<td>FAACL4</td>
<td>Xq22.3</td>
<td>Fatty-acid synthase-CoA ligase 4; possible role in membrane synthesis and/or recycling</td>
<td>Nonsyndromic XLID</td>
</tr>
</tbody>
</table>

continued
<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function of encoded protein</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC6A8</td>
<td>Xq28</td>
<td>Creatine transporter, role in homeostasis of creatine in the brain</td>
<td>Creatine deficiency syndrome and nonsyndromic ID</td>
</tr>
<tr>
<td>OCRL1</td>
<td>Xq25</td>
<td>Inositol polyphosphate 5-phosphatase (central domain) and RHoGAP-like C-terminal domain</td>
<td>Lowe syndrome</td>
</tr>
<tr>
<td>AGTR2</td>
<td>Xq24</td>
<td>Angiotensin II receptor type 2, signaling pathway</td>
<td>Nonsyndromic XLID</td>
</tr>
<tr>
<td>SLC16A2</td>
<td>Xq13.2</td>
<td>Monocarbohydrate transporter, T3 transporter</td>
<td>Severe syndromic form ID with abnormal levels of thyroid hormones</td>
</tr>
<tr>
<td>SMS</td>
<td>Xp22.1</td>
<td>Spermin synthase, CNS development/function (neuron excitability)</td>
<td>Snyder–Robinson syndrome</td>
</tr>
<tr>
<td>UBE3A</td>
<td>15q11</td>
<td>Ubiquitin-protein ligase E3A; protein degradation (proteasome); CNS development/function (neuron differentiation)</td>
<td>Angelman syndrome</td>
</tr>
</tbody>
</table>

Source: [11]
DNA which may extend for several hundred kilobases. Typically, the presence of repetitive DNA is restricted to the subtelomeric regions on a number of chromosomes [104]. The function, if any, of this subtelomeric repetitive DNA is unknown.

Naturally occurring mutations in humans show that chromosomes without subtelomeric repeats can be inherited normally, implying that the sequences have no important biological role [105]. However, sequence analysis of the DNA adjacent to the 4p, 16p, and 22q telomeres suggested that interstitial degenerate (TTAGGG)n repeats subdivide the subtelomeric regions into distal and proximal subdomains with different patterns of homology to other chromosome ends [104]. This view of the sequence organization of telomeres is shown in Figure 1.3. The interstitial (TTAGGG)n may have functional significance, serving to compartmentalize the subtelomeric domains in the nucleus, thereby limiting the nature and extent of sequence interactions. The size and complexity of the subtelomeric domains has made them extremely difficult to analyze at a molecular level. Subtelomeric sequence from one chromosome is frequently over 95% identical to the sequence from another and homologies often extend over many kilobases [101, 104]. Importantly, some subtelomeric sequences do include functional genes. The high degree of sequence similarity almost certainly reflects the action of frequent “cross talk” between telomeric regions, a process which may result in chromosomal rearrangements. Owing to the proximity to functional genes, rearrangements involving these regions are likely to have clinical consequences [106]. With the advent of the FISH technique, many investigators independently assessed and reported the frequency of subtelomeric rearrangements. Reported at an average of 6% [107], the range of estimated frequencies, however, vary widely [108]. Table 1.3 compares the frequencies reported across various studies.

Based on present knowledge, ID resulting from deregulation of genetic information might occur through (i) chromosomal rearrangements that lead to deleterious gene dosage effect, (ii) deregulation of the imprinting of specific genes or genome regions and (iii)
Figure 1.3: Sequence organization of telomeres and subtelomeric domains. Source: [109]

Table 1.3: Reported frequencies of Subtelomeric Rearrangements

<table>
<thead>
<tr>
<th>Technique used</th>
<th>Frequency detected (%)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypervariable DNA polymorphism</td>
<td>3</td>
<td>[97]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>23</td>
<td>[110]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>3.8</td>
<td>[111]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>7.4</td>
<td>[96]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>2.3</td>
<td>[112]</td>
</tr>
<tr>
<td>Microsatellite markers</td>
<td>7.5</td>
<td>[113]</td>
</tr>
<tr>
<td>Microsatellite markers</td>
<td>6.6</td>
<td>[114]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>0</td>
<td>[98]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>6.5</td>
<td>[115]</td>
</tr>
<tr>
<td>Multiprobe FISH + MAPH</td>
<td>1.4</td>
<td>[116]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>6.8</td>
<td>[117]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>5.1</td>
<td>[118]</td>
</tr>
<tr>
<td>CGH</td>
<td>2.8</td>
<td>[119]</td>
</tr>
<tr>
<td>CGH</td>
<td>29.4</td>
<td>[120]</td>
</tr>
<tr>
<td>Microsatellite markers</td>
<td>8.3</td>
<td>[121]</td>
</tr>
</tbody>
</table>

continued
Introduction

TABLE 1.3

<table>
<thead>
<tr>
<th>Technique used</th>
<th>Frequency detected (%)</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRINS</td>
<td>3</td>
<td>[122]</td>
</tr>
<tr>
<td>Microsatellite markers</td>
<td>10.7</td>
<td>[123]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>5</td>
<td>[124]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>9</td>
<td>[125]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>3.6</td>
<td>[126]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>4</td>
<td>[127]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>9.1</td>
<td>[128]</td>
</tr>
<tr>
<td>MLPA</td>
<td>4.3</td>
<td>[129]</td>
</tr>
<tr>
<td>MLPA</td>
<td>5</td>
<td>[130]</td>
</tr>
<tr>
<td>MLPA</td>
<td>2.9</td>
<td>[131]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>2.5</td>
<td>[108]</td>
</tr>
<tr>
<td>MLPA</td>
<td>3.9</td>
<td>[132]</td>
</tr>
<tr>
<td>Multiprobe FISH</td>
<td>13.6</td>
<td>[133]</td>
</tr>
<tr>
<td>MLPA</td>
<td>5.1</td>
<td>[134]</td>
</tr>
<tr>
<td>MLPA</td>
<td>0</td>
<td>[135]</td>
</tr>
<tr>
<td>MLPA</td>
<td>3.7</td>
<td>[136]</td>
</tr>
<tr>
<td>MLPA</td>
<td>4.2</td>
<td>[137]</td>
</tr>
<tr>
<td>MLPA</td>
<td>7.5</td>
<td>[138]</td>
</tr>
</tbody>
</table>

dysfunction of single genes (monogenic causes of ID), which are individually required for development of cognitive functions. ID resulting from these monogenic causes is either the only clinical manifestation of the disorders or a symptom of a clinical syndrome with or without detectable brain abnormalities. Some interesting observations with respect to the nature of the disease mechanisms have emerged from the identification of causative genes. First, there are a high number of genes that cause ID, far higher than predicted initially. Second, the brain, both in its development and maintenance, is exquisitely sensitive to alterations of gene expression and function. In fact, ID can result from abnormalities of genes that function in almost any biological process that the cell conducts. Genes that code for proteins that regulate global cellular functions such as DNA folding, chromatin remodelling and transcription factors as well as those that
are involved in structural components of post-synaptic membrane, protein trafficking, posttranslational modification of target proteins have all been implicated in the pathogenesis of ID [11].

The benefits of identifying genes that cause ID are many. From the research perspective, the knowledge could pave way for novel gene therapies that could be translated to patient care in future. In addition, knowledge about the cause, recurrence risks, short- and long-term prognosis, treatment options, availability of special services and support groups is of great importance to parents of affected children and often form the first step towards acceptance of the disability. Understanding that there is a gene mutation that causes the disease is of some therapeutic benefit in terms of preventing further unnecessary investigations in the child and in terms of resolving the uncertainty of not knowing the cause of disease. As ID is a life-long condition that cannot be cured, prevention is very important. Since the identification of a number of causative genes, parents come forth for predictive testing or have fewer children than predicted [139]. Also, from the healthcare perspective, the cost of a complete diagnostic work up for a child with ID is considerably huge and can be a major burden to the public health system.

1.6 Evaluation

The principal objectives of medical evaluation in a child with ID/GDD are to (i) confirm the precise neurodevelopmental disability thorough history, assessments and appropriate laboratory testing, (ii) identify and arrange for needed supports and interventions, (iii) counsel the family regarding the disability, including recurrence risks, and (iv) identify conditions that may require specific interventions to optimize the realization of the child’s full developmental potential [1]. Thus, the evaluation of the child with ID involves a multidisciplinary approach with psychological, clinical and genetic components; the essential elements of evaluation include a three-generation pedigree, prenatal, perinatal and postnatal history, in depth physical examination focused on minor anomalies, neurologic examination and assessment of the behavioral phenotype where
indicated. Figure 1.4 outlines the recommended approach to the evaluation of a child with ID.

1.6.1 Family history

A careful and detailed history that covers three generations and uses open-ended questions regarding the health and developmental status of family members is fundamental to the evaluation of ID/GDD. Ethnic heritage and geographic origin are essential information as is the possibility of parental consanguinity or previous familial neonatal/infantile deaths or maternal pregnancy losses. The mother, who is the likely provider of all the necessary information must be queried about adverse antenatal events, maternal prescription medication use or substance abuse, timing of labor, mode of delivery, and any adverse events during the delivery process. Then, details of the child’s birth weight; appearance, pulse, grimace, activity, respiration (APGAR) scores; duration of infant’s postnatal stay and the occurrence of any neurological symptoms as a newborn provide important clues to time the origin of a child’s developmental disability. Postnatal history includes information concerning hospital admissions, surgical procedures, chronic medical conditions and medication use. The child must also be examined for coexisting autistic features and behavioral concerns. The socioeconomic status of the parents and the child’s psychosocial environment also need to be documented. With respect to the child’s developmental history, the age at which parental concern regarding developmental difficulties arose should be elicited by inquiring about key motor and language milestones. With sufficient background, the child’s developmental history may be placed into individual, familial and social context and the loss of functional or developmental skills may be determined [1].

1.6.2 Psychological assessment

Various types of standardized psychological tests are used to assess IQ, learning abilities, and behavioral skills. IQ tests have two parts: one to measure verbal abilities, and the other to measure spatial abilities, which refers to movement and manipulation in
FIGURE 1.4: Current practice of evaluating a subject with ID. Source: [140]

Cytogenetic and Molecular Genetic Evaluation of Genomic Rearrangements in Children with Intellectual Disability
three-dimensional space. In general, the IQ scores are standardized and the test is uniformly designed and consistently administered, which allows comparisons of individual scores against average scores for the same group. This provides vital information about a person’s skills and abilities relative to their peers. IQ tests measure reasoning, problem solving, abstract thinking, judgment, academic learning, and experiential learning. Tests of adaptive functioning skills are used to evaluate the social and emotional maturity of a child and thus, life skills and abilities. The Vineland adaptive behavior scale (VABS) is a test to measure social skills including communication, daily living skills, socialization and motor skills. The Gesell Children’s Behaviour Schedule (GCBS) is another test measuring the development in infants and young children, expressed in the form of developmental quotient.

1.6.3 Clinical evaluation

The clinical evaluation consists of physical and neurologic examinations. General physical examination includes measurement of height and weight and comparing the relevant percentiles with the appropriate reference norms with the available population data. A thorough physical examination for minor anomalies that might suggest an etiology or contribute to the recognition of a particular diagnostic pattern is fundamental to the evaluation. These features need to be considered within the context of familial and ethnic variation.

1.6.3.1 Dysmorphologic examination

In general, a dysmorphologic examination includes assessment of (i) the skull for shape and size, shape of fontanelle and hair texture and distribution, (ii) eyelashes and eyebrows for shape, distance between outer and inner canthi, presence of epicanthal folds, pupil dilation, iris coloration and patterning and presence of cataract, (iii) the nasal bridge, shape and size of nose and lips, size of the philtrum as well as the position and placing of teeth and palate, (iv) the position of the ears relative to the angle of the jaw, (v) presence of webbing or excessive nuchal skinfolds in the neck, (vi) the shape of
the chest and spacing of nipples, in addition to heart sounds and breathing, (vii) the abdomen for masses or enlargement of organs and the external genitalia for abnormalities, (viii) upper and lower limbs for proportion and range of motion, (ix) hands for palmar creases, fingers, toes and nails for number, length, shape and position and (x) the skin for texture, pigmentation, café-au-lait spots, skin tags, elasticity and vascular lesions [85]. Figure 1.5 provides photographic descriptions of a few of the dysmorphic features documented in various publications. Although the evaluation of dysmorphic features does not narrow down a clear etiology or indicate the reason to initiate further metabolic, cytogenetic, molecular, neuroradiological or other investigations, it has been emphasized that a detailed physical examination by a trained specialist remains the basis of every etiological study in children with ID [143].

1.6.3.2 Neurodevelopmental assessment

It is a large component of clinical evaluation, most of which is made by observation. The key element is obtaining the occipital frontal head circumference, and deriving age- and gender-appropriate percentile to document macrocephaly or microcephaly in the child. Following which, the cranial nerves are assessed for visual defects, facial paresis, speech difficulties, among others. The muscle bulk, strength, tone, reflexes and plantar responses as well as limb movements and gait are assessed for the motor component [1]. In the presence of seizures in the child, an electroencephalogram (EEG) may be recommended. The significance of neuroimaging studies in the evaluation of children with ID is debated between their application as second-line tests and the validity of performing them on every affected individual. The finding of a brain abnormality or anomaly on neuroimaging may offer a useful diagnosis; however, the diagnostic yield based on neuroimaging varies widely in the literature owing to subject selection and method of imaging used. Earlier studies using computerized tomography (CT) reported poor diagnostic yield [146], while more recent reports based on magnetic resonance imaging (MRI) showed a better diagnostic yield [143, 147]. It certainly contributes to higher
Introduction

**Figure 1.5**: Representative images of a few dysmorphic features.
*Source: [144]*

---

**A. Abnormalities of the head**

- **Microcephaly (small head)**
- **Macrocephaly (large head)**
- **Brachycephaly (flat occiput)**

**B. Abnormalities of the eyes**

- **Hypertelorism (increased intercanthal distance)**
- **Bilateral ptosis of eyelids**
- **Upslanted palpebral fissures**
- **Downslanted palpebral fissures**

**C. Abnormalities of the skin**

- **Hyper- and Hypopigmentation**
- **Hyperelasticity of skin**
D. Abnormalities of the ear and mouth

- Low-set, posteriorly rotated ear
- Macrostomia (big mouth)
- Short philtrum
- Micrognathia (small mandible)

E. Abnormalities of the limbs

- Brachydactyly (short fingers)
- Arachnodactyly (long fingers)
- 5th finger clinodactyly (incurved fingers)
- Simian Crease
- Rocker bottom feet
diagnostic yields when concurrent neurologic indications exist, derived from a careful physical examination [148].

1.6.4 Genetic evaluation

Based on a detailed medical history, clinical and psychological assessments, selective laboratory testing is the next logical step in the evaluation of the child with ID, which would lead the investigation towards the specific etiology. Available guidelines recommend that banded karyotype analyses, assessment for fragile X and metabolic studies be performed based on information obtained from history and clinical examination [149]. The benefits of appropriate genetic evaluation for the patient are diverse and include (i) identification of specific therapies, (ii) presymptomatic screening for associated complications, (iii) educational planning, and (iv) elimination of unnecessary and expensive testing. Furthermore, for the parents/family, they include (i) anticipatory guidance, (ii) education and advocacy, (iii) referral to medical and social service agencies and support groups, (iv) reproductive counseling, carrier testing and prenatal diagnosis, and (v) family networking [149]. Chromosome analysis in the evaluation of children with ID/DD is recommended in all children for whom the etiology is unknown. There are many developments in the techniques employed to screen and detect abnormal genes and/or gene loci and evaluate their association with ID as discussed further.

1.6.4.1 Cytogenetic methods

1.6.4.1.1 Chromosome analysis by GTG banding

Conventional cytogenetic (GTG banding) analysis can detect gross chromosome abnormalities, but is limited by its resolution in the detection of subtle chromosomal rearrangements in the order of a few thousand base pairs. The use of early metaphase or prometaphase chromosomes for GTG banding results in bands of the order >550 bands per haploid set being observed [150]. Submicroscopic rearrangements that are invisible at the 450-band level can be detected using High Resolution Banding (HRB). It is a
simple technique that is cost-effective and allows for preliminary testing of submicroscopic rearrangements. Depending on the variation in the study design among published reports, a wide range (2% to 50%) of reported frequencies of chromosome abnormalities causing ID is observed. Reports show that chromosome abnormalities are found in all categories of ID (mild to profound) and in both genders [143]. Shevell et al [3] noted that routine cytogenetic testing is indicated in the evaluation of the child with ID/DD even in the absence of dysmorphic features or clinical features suggestive of a syndrome. Curry et al [149] stated that chromosome analysis in the individual with ID is generally regarded as a mainstay in the overall evaluation process. Despite the fact that the G-banded karyotyping has been the standard first-tier test to detect genomic imbalance in children with ID/GDD worldwide for the past 35 years, it is, of late, recommended that it may be replaced by chromosome microarray (CMA) as a first-tier diagnostic test in all children with ID/GDD with an unknown cause [148]. However, the cost of such screening for every patient will be huge, and may not become the norm in the developing world for a few years to come.

1.6.4.1.2 Fluorescence in situ Hybridization (FISH)

FISH is a sensitive technique using short DNA probes (fluorescently conjugated complementary sequences to target DNA) to detect the presence of specific segments of the genome. This technique has the advantage of detecting deletions and balanced or unbalanced translocations. Perhaps a disadvantage of FISH is that it is labor-intensive and time-consuming. In the evaluation of the child with ID/GDD, FISH may be employed to investigate particular regions of the genome. Since a complete set of FISH probes for subtelomeres has become available clinically [151, 152], the utility of these probes has been demonstrated by numerous reports of patients with ID who have had a normal routine karyotype previously [96, 107]. The yield of targeted FISH studies to identify the cause of ID vary among reports and depends on various factors such as clinical presentation, subject selection, and variability of expression.
1.6.4.1.3 Chromosome Microarray (CMA)

CMA enables the analysis of the genome for chromosomal rearrangements or genomic imbalances, including chromosomal gains and losses at high resolution and sensitivity [153]. The primary advantage of CMA over the standard karyotype or FISH techniques is its ability to detect DNA copy changes simultaneously at multiple loci in a genome in a single experiment or test. CMA techniques or “platforms” vary and the arrays are either for whole-genome, which are designed to cover the entire genome, or partial genome, which target known pathologic loci. CMA encompasses all types of array-based genomic copy number analyses, including array-based comparative genomic hybridization and single-nucleotide polymorphism arrays [153]. The rate of diagnosis by CMA of patients presenting with ID/GDD depends upon the type of microarray technique [154]. Nevertheless, the diagnostic yield for all current CMA is estimated at 12% for patients with GDD/ID [148].

1.6.4.2 Molecular genetic methods

1.6.4.2.1 DNA markers

Along with developments in the cytogenetic methods, molecular methods that probe specific regions of the genome have been developed. Slavotinek et al [113] screened 27 subjects with ID for submicroscopic rearrangements using microsatellite markers from the subtelomeric regions of 41 chromosome arms. The use of genetic marker analysis allows the determination of the physical size of deletions and duplications and the parent of origin. They reported a detection rate of 7.5% and an abnormality frequency of 18%. Although the technique is inexpensive and easy, it requires parental DNA samples as well as markers with high informativeness [114].

1.6.4.2.2 Gene-specific Polymerase Chain Reaction

In children with ID/GDD suspected to follow a Mendelian principle, a simple gene-specific Polymerase Chain Reaction (PCR) is informative. Various methodologies have
been proposed that use PCR followed by sequencing for the analysis of single genes such as \textit{FMR1} and \textit{MECP2}, mutations in which result in ID [155–157]. It is recommended that \textit{FMR1} gene screening is performed in all individuals without a known cause of ID [148]. It is estimated that 2% to 3% of boys with ID/GDD of uncertain cause will have FXS, whereas, in girls it is 1% to 2% [158]. Rett syndrome is an X-linked condition that affects girls and results from \textit{MECP2} gene mutations primarily; many studies have examined the rate of pathogenic \textit{MECP2} mutations in children with ID. The proportion of \textit{MECP2} mutations in these series ranged from 0% to 4.4% with an average of 1.5% among girls with moderate to severe ID [159, 160].

1.6.4.2.3 Quantitative real-time PCR

Quantitative real-time PCR (qPCR) is a sensitive method capable of quantitative genotyping of amplicons using SYBR-green dye. This technique allows high-resolution screening of single copy number gains and losses by their relative quantification against a diploid genome [161]. Auber et al [162] reported a frequency of 5.7% of subtelomeric alterations in a group of 296 individuals with ID with primers designed for every telomere. It is a labor-intensive method; however, it offers the potential to characterize the size of genomic imbalance in a cost-effective manner.

1.6.4.2.4 Multiplex Amplifiable Probe Hybridzation

Multiplex Amplifiable Probe Hybridization (MAPH) is a novel technique that can detect and define a large series of new mutations—in particular, duplications—with several not detected by Southern blotting and/or qPCR [163]. The method uses oligonucleotide probes that hybridize to specific nucleic acid sequences. Each hybridized probe can be simultaneously amplified with the use of a single primer pair and yields an amplification product of unique size. The copy number of target sequences is reflected in the relative intensities of the MAPH probe amplification products [164]. However, like Southern blotting, MAPH requires immobilization of sample nucleic acids and tedious washing of unbound (amplifiable) probes, making it difficult to implement in a routine diagnostic
Introduction

Despite the potential for extension to genome-wide screens for deletions and amplifications, the methodological constraints limit the utility of the technique.

1.6.4.2.5 Multiplex fluorescence PCR

Following the development of the MAPH technique, a related yet simpler method was reported by Schouten et al [165], called Multiplex Ligation-dependent Probe Amplification (MLPA). In MLPA, probes added to the samples (not sample nucleic acids) are amplified and quantified. Amplification of probes by PCR depends on the presence of probe target sequences in the sample. A number of studies have employed MLPA using various panels for subtelomeric alterations, interstitial microdeletions, and X-linked ID. MLPA is a relatively inexpensive and easy technique in comparison to CMA and offers potential for the screening of children with ID. Published reports suggest a range of 2.9% - 5.9% of subtelomeric abnormalities detected in children with ID using MLPA [138]. Saugier-Veber et al [166] reported a novel multiplex quantitative-fluorescence technique, quantitative multiplex PCR of short fluorescent fragments (QMPSF) to detect genomic imbalances in ID. They reported an abnormality rate of 1% in a screening of genomic hot spot regions in children with ID.

1.6.4.2.6 Next Generation Sequencing

The development of the next generation sequencing (NGS) platforms have revolutionized the field of genomics in the recent years. Sequencing of protein-coding parts of the genome, known as exome sequencing, offers great diagnostic potential as it is a more feasible and faster option in comparison to whole genome sequencing. A number of studies have employed massively parallel sequencing in specific ID-related conditions to identify the candidate genes [167]. Recently, Tarpey et al [168] reported the identification of nine novel X-linked ID-associated genes and pathogenic sequence variants in 17% of the cohort families. It is likely that many more disease genes with functional significance will be discovered in the future.
Thus, the scrutiny of literature demonstrated rapid developments in the technology towards identifying the underlying cause in ID. Nonetheless, each technique has its advantages as well as demerits. Table 1.4 compares the available techniques which are currently being used for the genetic evaluation of ID.

<table>
<thead>
<tr>
<th>Technique for genetic evaluation</th>
<th>Merits of the technique</th>
<th>Demerits of the technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTG banded karyotyping</td>
<td>Whole genome approach</td>
<td>Limited resolution</td>
</tr>
<tr>
<td>High resolution banding (HRB)</td>
<td>Whole genome approach</td>
<td>Target-specific probing for confirmation</td>
</tr>
<tr>
<td>Single gene mutation analysis</td>
<td>Higher resolution relative to conventional banding</td>
<td>Confined to specific region</td>
</tr>
<tr>
<td>Fluorescence in situ Hybridization (FISH)</td>
<td>Highly sensitive</td>
<td>Targeted approach</td>
</tr>
<tr>
<td></td>
<td>Detects balanced rearrangements</td>
<td></td>
</tr>
<tr>
<td>Multiplex Ligation-dependent Probe Amplification (MLPA)</td>
<td>Rapid and sensitive</td>
<td>Targeted approach</td>
</tr>
<tr>
<td></td>
<td>High throughput</td>
<td>Cannot detect balanced rearrangements</td>
</tr>
<tr>
<td></td>
<td>Cost effective</td>
<td></td>
</tr>
<tr>
<td>Multiplex Amplifiable Probe Hybridization (MAPH)</td>
<td>Sensitive</td>
<td>Labor-intensive</td>
</tr>
<tr>
<td></td>
<td>Detection of new mutations</td>
<td>Cumbersome</td>
</tr>
<tr>
<td>Quantitative Multiplex PCR of Short Fluorescence fragments (QMPSF)</td>
<td>Multiple loci screened simultaneously</td>
<td>Targeted approach</td>
</tr>
<tr>
<td>Array Comparative Genomic Hybridization (aCGH)</td>
<td>Genome-wide analysis at high resolution</td>
<td>Expensive</td>
</tr>
<tr>
<td></td>
<td>Potential for rapid screening</td>
<td>Cannot detect balanced rearrangements</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caution in data interpretation</td>
</tr>
</tbody>
</table>
1.7 Management of ID

In all cases of ID, the crux of treatment is early detection and intervention aimed at minimizing the disability through reducing risks. An etiological treatment is possible in some cases of ID, where a metabolic cause is determined. Medication is more often incorporated in the management regime for neurological and psychological disturbances. As children with ID have difficulty learning, tailor-made education programs enable better coping for children rather than segregating them into special schools. This has already been implemented in developed countries, but may take time to be adapted into the education system of developing countries [169]. Overall, attending school is essential for children with ID to learn not only academic skills but also self-discipline, social and practical skills for community living. Physical therapy and occupational therapy contribute to management, as ID is often accompanied by poor muscle tone, lack of coordination and slow development of motor skills. Speech therapy has been shown to effectively promote speech, language and communication ability. Also, augmentative and alternative communication strategies are suggested to help children with all levels of ID [170]. In addition to care of the affected child, family support and education are essential. The WHO suggests that family support needs to include communication of the diagnosis and information about it, emotional support, family counselling and training, involvement in health care decisions and respite care [171].

1.8 Hypothesis

The evaluation of a heterogeneous disorder such as ID is complex, and various genetic components may play an etiological role in the pathogenesis of the condition. Newer tools developed to explore the genome provide enhanced knowledge, at least in understanding the etiology of ID and its management. Existing literature reports submicroscopic genomic rearrangements as significant causative factors of idiopathic ID. However, reported frequencies vary widely among studies, necessitating more information from different populations to reach a consensus. Also, there is a dearth of data in India.
In addition to a thorough account of the history and clinical evaluation, comprehensive genetic testing is paramount to establishing the etiology. A multipronged approach to the assessment of genetic factors will aid in better etiological diagnosis of ID and lead to characterization of ID-syndromes. Based on the hypothesis, the aim and objectives of the thesis are derived as follows.

1.9 Aim of the thesis

This thesis aims to investigate the role of genetic factors in the pathogenesis of idiopathic intellectual disability.

1.10 Objectives

- To establish the cause of intellectual disability in a south Indian population of children through cytogenetic evaluation and fragile X screening
- To investigate subtelomeric rearrangements and estimate their frequency by FISH
- To study interstitial microdeletions and microduplications using MLPA and QMPSF
- To characterize genes present at specific loci and estimate the extent of their rearrangement in subjects with terminal deletions and duplications
References


Introduction


Introduction


Introduction


Introduction


Introduction


Introduction


Introduction


Introduction


Introduction


Introduction


