SECTION 1

REVIEW OF LITERATURE ON DEVELOPMENTAL DYSLEXIA
Language is a communicating system that uses sounds or symbols to express thoughts and ideas. The capacity to acquire and use of language is a key aspect that distinguishes humans from other beings (Tomasello, 2008). Reading and writing are basic life skills and essential part of language, which are cornerstone for a child's success in school and indeed throughout life. Most individuals can acquire the ability to read and write words accurately or fluently but a significant percent of children faces difficulty in these tasks. The unexpected difficulty in reading and writing despite having adequate intelligence, education and social environment is called as Developmental Dyslexia (DD) (World Health Organization, 1993). DD is generally referred as specific impairment in reading ability that is substantially below the expected reading ability given the person’s chronological age, measured intelligence and age-appropriate education. Research on DD was initiated on 19th century and it was known as ‘word-blindness’. Later this was named as ‘dyslexia’ the word derives from two Greek words ‘Dys’ meaning ‘lack of’ or ‘difficulty’ and ‘Lexis’ meaning ‘word’.

DD primarily affects the skills involved in accurate and fluent word reading and spelling. Letter and number reversals are the most common warning sign. Along with this difficulties can be observe in phonological coding, memory, encoding, retrieving and using (Catts, 1989). Language processing, understanding the words, forming sentences, poor verbal Short Term Memory (STM) and limited speech sound awareness is usually observed in individuals with DD (Bradley and Bryant, 1983; Wolf and Bowers, 1999). DD shows the co-morbidity with several other disorders, particularly with neuro-developmental disorder, such as Attention-Deficit Hyperactivity Disorder (ADHD) and Specific Language Impairment (SLI) (Shaywitz, 1998; Pennington and Bishop, 2009).
ADHD was observed in 25-40% of children with DD (Dykman and Ackerman, 1991) and in adolescence depressive disorders and disorders of social behaviour are often associated with DD (Eissa, 2010).

1.1 Epidemiology

Epidemiological studies have identified the frequency of distribution of DD in different populations. The distribution of the disorder varies in different populations according to the selection criteria and test languages used. In US, a prevalence rate of 5.3 - 11.8% was figured out by investigating 5,718 children in a population based birth cohort (Katusic et al., 2001). In Chinese population, study of 690 children in Hong Kong showed prevalence rates of 9.7–12.6% (DeFries and Alacron, 1996) and a recent study in China revealed a prevalence rate of 3.9% by investigating 5,063 students (Sun et al., 2013). In Egypt, prevalence rate was 1.3% (Farrag et al., 1988) and in Finnish adults sample it was about 6% (Lytyinen et al. 1995). The prevalence of dyslexia and probable dyslexia were found to be 6.3 % and 12.6 % in Thailand (Roongpraiwan et al., 2002). In Italian population it has been ranged from 3.1% to 3.2% depending on different criteria adopted (Barbiero et al., 2012). In monolingual Persian students 5.2% of the prevalence was observed (Pouretemad et al., 2011). In South India, it was reported to be 9.87 % among school children of South India and 28.32% among selected families (Saviour et al., 2009). In Bikaner City of Rajasthan, a survey of 468 students showed a prevalence of 10.25% (Choudhary et al., 2012). Measuring the prevalence of specific learning disabilities such as dyslexia, dysgraphia and dyscalculia among primary school
children in a South Indian city showed a prevalence of 11.2% for dyslexia (Mogasale et al., 2012).

Prevalence of risk of DD among boy and girls was a controversial issue. It was found to be higher in males compare to females (Rutter et al., 2004). Some studies have also showed that the risk is double in boys compared to girls (Katusic et al., 2001; Roongpraiwan et al., 2002; Chan et al., 2007). However, in some studies, ratio of inheritance of risk is equal in males and females (Harlaar et al., 2005; Hawke et al., 2006). Epidemiology study of four independent samples n = 989, 895, 5,752 and 2,163 showed prevalence of 18.5–24.6% in boys and 8.3–13% in girls. It has been also proved by analyzing 32,223 children that, risk is twice in boys compare to girls (Flannery et al., 2000). A survey in 43 different countries also showed unexpected outperformance of girls than boys on reading tests (Chiu et al., 2006). Studies in Asian countries namely India and China showed a higher rate of prevalence in boys compare to girls (Saviour et al., 2009; Choudhary et al., 2012; Sun et al., 2013).

1.2 Phenotypic classification

The phenotypes of DD can be grouped broadly into poor reading/decoding, poor phonological skills, poor Rapid Automatized Naming (RAN), and poor working memory. There is no definite information on the co-occurrence rate of these different phenotypes. However, poor reading performance is a must in DD and most of the studies also suggest poor phonological skills as the basic deficit often in combination with other features mentioned. Reading and spelling involves many complex cognitive components, below mentioned are such processes include in reading and writing (Schumacher et al., 2007).
**Visual processing:** The magnocellular system responds to moving stimuli and stimuli of low spatial frequency and low contrast. Impaired perception of moving stimuli and the neurophysiological correlates of this have been found repeatedly in individuals with dyslexia.

**Phonological awareness:** The ability to perceive, segment and manipulate the sounds of spoken words. Phonemes are the smallest meaningfully distinct sounds from which an acoustic speech flow can be constructed.

**Verbal STM:** Various aspects of memory are required for reading. Many known words are no longer dissected into their phonemes, but are recalled directly from memory. Processing of unknown words into their phonemes occurs in STM. STM is often examined by a digit span task.

**Phonological coding:** The ability to put together the phonemes and then verbally express words which have never been previously read or heard. This ability is tested through reading of pseudowords.

**Orthographic coding:** Orthographic coding refers to the assumed process of recognising a word by its holistic form. Orthographic coding is measured by a pseudohomophone task where an orally presented word has to be compared with a visual presentation of two phonologically indistinguishable words, of which, one may be orthographically correct.

**Rapid naming:** Rapid naming is a measure of the speed of processing. The naming of objects, numbers, letters and colours is typically measured (Schumacher *et al.*, 2007).
1.3 Diagnosis and assessment

When assigning a qualitative DD phenotype, individuals are commonly classified as affected if test scores reveal a reading ability that is two years below the expected chronological age despite normal verbal IQ (Williams and O’Donovan, 2006). The usual assessment procedure involves a test of general intellectual abilities using standardised objective tests, to determine the adequacy of operation of other mental capacities. The results of these tests are used as a baseline for evaluation of any discrepancy between that and achievement in reading, spelling or writing (Temple et al., 2003; Ramus, 2004).

The necessary test materials required to diagnose and identify children with DD are:

1. Standard Progressive Matrices (SPM)

   SPM is a non verbal multiple choice test which measures general intelligence, appropriate for ages 8-65 years. SPM test consists of 60 problems and it has been divided into five sets A, B, C, D and E. Each set consists of 12 problems and the subject is asked to identify the missing element or part that completes a pattern (Raven, 1936).

2. Phonological awareness tasks

   Phonological awareness refers to an individual's ability to detect and manipulate sounds at three levels, rimes, syllables and phonemes. Rhyme generation task consists of ten items. Children are required to listen to word and generate another word which rhymes. Syllable task consists of syllable deletion and syllable reversal. Both the tasks consist of ten items. In syllable deletion, children were required to delete a particular syllable and report the remained word. In syllable reversal tasks children were required to reverse the syllable and report it. In scoring no point is given to each correct response.
Phoneme task consists of phoneme deletion and phoneme reversal. Both the task consists of ten items. In phoneme deletion task children were required to delete an individual sound for the word presented and to report. In phoneme reversal children were instructed to listen to the orally presented words carefully and repeat them backwards phonemically. The correct response would always a non word. The responses will be recorded in the scoring sheet.

3. RAN

RAN consists of four sections, letter naming, digit naming, object naming and colour naming. The letters, numbers, colors, and objects tests are made up of five high frequency stimuli that are repeated randomly 10 times in an array of five rows for a total of fifty stimulus items. Scores are based on the amount of time that is required to name all stimuli on each test (Denckla and Rudel, 1974).

4. Word Reading Test

The English word reading and spelling list (Joshi and Aaron, 2003) has 56 words and the words are categorized into four groups viz, regular words, exception words, unique words and morphophonemic words. The child has to read the list of words horizontally and correctly and has to read as many words as possible. Total time taken to complete the task was noted down and the response was recorded. Later correct response and the wrong response was calculated. Every word read correctly will get a score.

5. Digit forward and backward tests (Post Graduate Institute Memory Scale (PGIMS))

The PGIMS was employed to assess memory function of cases. PGIMS consists of ten sub-tests to measure different aspects of memory and employ different methods of
recall. In digit forward task, subjects were asked to repeat the number as it is heard where in digit backward subjects, were asked to repeat the number in backwards. Manual was used to do the scoring (Pershad and Wig, 1976).

6. **Edinburgh test of Handedness**

   Edinburgh test of handedness was used to assess whether the child is right handed, left handed or mixed handed. This test consists of ten items. Subjects were asked which hand they use while doing some particular task like writing, throwing ball etc (Oldfield, 1971).

7. **Bender Gestalt Test (BGT)**

   BGT is a psychological test, developed by child neuropsychiatrist Lauretta Bender. The test was used to evaluate to screen for developmental disorders. The original test consists of nine figures, each on its own 3 × 5 card. The subject is shown each figure one after one and asked to copy it onto a piece of blank paper. Administration of the test takes approximately 7–15 minutes, after which the results are scored based on accuracy and other characteristics.

8. **Non-word reading tests**

   A list of non words in English was prepared, out of which, only 56 words were selected. The child has to read the list of words and total time taken to complete the task was noted down. Responses were recorded in a voice recorder, later correct response and wrong responses given by a subject were marked in a response sheet. Each correct response was given 1 mark and zero for the wrong one.
1.4 Family history

Evidence for the heritability of DD was obtained in the middle of 19th century by studying a large-scale family (Hallgren et al., 1950). Familial segregation and twin studies have provided consistent evidence for the significant role of genetic factors in the etiology of DD (Hallgren, 1950; Pennington et al., 1991; Farrer, 2004; DeFries and Alacron, 1996). Colorado-based study suggests concordance of trait was 68% in monozygotic twins whereas, in dizygotic twins, it is 38% suggestive of a genetic aetiology for DD (DeFries and Alacron, 1996). Another twin study also reported significant variation between monozygotic and dizygotic twins, concordance of the trait in monozygotic twins is higher compare to the dizygotic twins because of genetic identity (Farrer et al., 2004). Familial factors play a major role in manifestation of DD. Risk of DD increase with the presence of other affected family members (Hallgren, 1950). Comparison of the familial history of children with DD and controls showed, 34% children with DD, 9% of the controls having a sibling or parent affected with DD (Rutter and Yule, 1975). There are 50% of chances of occurring DD from parents to the children and 50% chances of occurrence of risk in sibs (Finucci et al., 1976). If both the parents are affected risk of DD increases to 76–78% and if single parent is affected the risk of DD is 54–63% more compare to the unaffected parents (Gilger et al., 1996). It has been also proved that there are 20–33% siblings of children with DD were affected even though their parents are unaffected (Gilger et al., 1996). All these studies on family history of DD proved that frequency of risk of appearance of the disorder is more if parents or siblings are affected. Though the risk was observed in the family members of the control samples it was less compare to the families of children with DD.
Pattern of inheritance of DD is still controversial and initially by investigation of 112 families with DD Hallgren et al., (1950) identified 80% families with autosomal dominant pattern of inheritance. Sex influenced autosomal dominant pattern of inheritance was reported by Zahalkova et al. (1972). Later, Pennington et al., (1991) study found the evidences for autosomal dominance pattern of inheritance, which was verified for only 20% of the families. Finucci et al., (1976) reported that DD have more than one mode of transmission, either autosomal dominant, autosomal recessive, or multifactorial inheritance based on the complexities associated with specifics learning disability. Studies have also reported that there was no evidence for the single major locus, autosomal dominant, recessive or codominant transmission (Lewitter et al., 1980; Grigorenko et al., 1997). By these results, it was confirmed that DD a complex disorder which does not follow the classical Mendelian pattern of inheritance (Grigorenko et al., 1997). In Indian population analysis pedigrees of children with DD revealed 82% of the autosomal dominant pattern inheritance associated with reading spelling deficit (Saviour et al., 2009).

1.5 Etiology

It has often been observed that DD runs in families, and evidence from family and twin studies have shown that genetic factors do play a role and many candidate genes have been identified to be associated with DD (DeFries and Alacron, 1996). Genes do not work in isolation; environmental experiences will have an influence on the impact of genes and the severity of difficulties. A number of theories have been proposed regarding the cognitive causes of DD (Ramus et al., 2003).
1.5.1 The phonological deficit theory

This is the most accepted theory which explains that reading a word includes dividing the word into its underlying phonemes (Shaywitz and Shaywitz, 2003). The difficulty in the use of language is mainly due to the problem in interaction of storing, perception and motor output. Storing of language requires some basic skills like phonological and orthographical awareness (Price, 2000). Apart from this, understanding the words and connecting the words in sentences is engaged by the nervous system. To understand and speak a word it is very important to retrieves the word’s phonemic constituents, assembles the phonemes, and complete the word (Shaywitz and Shaywitz, 2003). Individuals with DD have problem in phonological processing. Most evidence suggests that deficits in DD are mainly due to the difficulty in perceiving and segmenting phonemes which leads to the poor reading and difficulty in creating connection between phoneme and grapheme (Shaywitz et al., 1999; Ramus et al., 2003). Poor performance of children with DD on phonological tasks like verbal STM, automatic naming, segmentation and manipulation of speech sounds provided the support for phonological theory (Snowling, 2000).

1.5.2 The cerebellar theory

Cerebellum of brain involves in many tasks viz. sensory perception, automatization of tasks and skills, as well as the motor output. Impairments of the cerebellum cause deficits in motor skills such as posture, balance and week automation results in deficient phonological representations and affects reading related areas like grapheme-phoneme correspondences. This theory was supported by the studies which showed that poor performance of dyslexics in a large number of motor tasks, in dual tasks
demonstrating impaired automatization of balance and in time estimation, a non-motor cerebellar task (Nicolson and Fawcett, 1990; Nicolson et al., 1995; Fawcett et al., 1996).

1.5.3 The magnocellular theory

Auditory and visual processing for spoken and written words plays a major role in phonological and orthographic storing (Price, 2000). DD has been associated with deficit in visual and auditory processing (Livingstone et al., 1991; Stein and Walsh, 1997). This theory explains that DD is due to the auditory deficit and visual impairment. Auditory deficit prevents the perception of short and rapidly varying sounds (Tallal, 1980). Visual impairment is associated with Central Nervous System (CNS) with impaired sensitivity of cells within the retinocortical magnocellular pathway. Dysfunctions of cells in the magnocellular pathway affect all sensory modes and also the posterior parietal cortex and the cerebellum (Stein and Walsh, 1997). This theory also explains that dysfunctioning of magnocellular is not only restricted to visual pathway but also associated with auditory and tactile. Cerebellum receives the input from the magnocellular pathway it is predicted that dysfunctioning of magnocellular pathway affect the input to cerebellum (Stein et al., 2001; Benitez-Burraco, 2010).

1.5.4 Other theories

One more theory was proposed to explain the deficit in reading which is named as rapid auditory processing theory. This theory postulates that deficit in the perception of various auditory sounds (Tallal, 1980; Tallal et al., 1993). Poor performance of children with DD on auditory tasks like, frequency discrimination and temporal order judgment provided the additional support for this theory (Tallal, 1980; McAnally and Stein, 1996; Nagarajan et al., 1999, Ahissar et al., 2000; Rumus et al., 2003). Failure in the pursuing
the short sounds causes the phonological deficit thereby difficulty in learning to read (Mody et al., 1997; Adlard and Hazan, 1998; Serniclaes et al., 2001). Another theory named as visual theory explains that, difficulty in processing the letters and word is due to the visual impairment (Cornelissen et al., 1993; Stein and Fowler, 1993; Eden et al., 1994). Selective disruption of magnocellular pathway in children with DD leads to the deficit in visual processing and, via the posterior parietal cortex, to abnormal binocular control and visuospatial attention (Stein and Walsh, 1997; Hari et al., 2001). All these theories accounts for different sub-sets of DD to find the different etiology for DD by combining neurological and environmental factors.

1.5.5 Neurobiological studies

Brain imaging and postmortem study of brain provided supporting evidence for these theories by observing abnormality in the brain of children with DD. These abnormalities include increase in the left hemisphere concentrated around the perisylvian region and near symmetry of the planum temporal. Postmortem report of visual processing revealed disorganization and reduction in the size of the magnocellular layers of the Lateral Geniculate Nuclei (LGN). This region of the brain involved in the visual system and in the cases of DD disorganization of these cells indicates the visual processing deficiency in DD (Galaburda et al., 1979; Galaburda et al., 1985; Humphreys et al., 1990; Livingstone et al., 1991).

Subsequently, DD cases with rapid auditory processing were examined for the Medial Geniculate Nuclei (MGN) which is involved in the auditory processing system. This experiment revealed the presence of smaller neurons and greater asymmetry between the left and right MGN compare to the control brains (Galaburda et al., 1979;
Galaburda et al., 1985; Humphreys et al., 1990). In fMRI study of dyslexics and non-dyslexic observed a difference in the activation of brain pattern in reading of non-words (Fig. 1.1). Dyslexic children showed greater activation in left hemisphere sites such as the parieto-temporal and middle temporal or middle occipito-temporal region (Shaywitz et al. 2002). Further functional studies also supported that disruptions of parieto-temporal and occipitotemporal left hemisphere are the reason for lack in the development of reading skills in children with DD (Paulesu et al., 1996; Salmelin et al., 2000; McCrory et al., 2005; Dufor et al., 2007; Wehner et al., 2007). The under-activated these two regions of the left hemisphere is used as ‘neural signature for dyslexia’ (Shaywitz and Shaywitz, 2008).

1.5.6 Biological Factors

Emerging body of evidences suggest that, biochemical factors also play a major role in manifestation of DD. The Polyunsaturated Fatty Acids (PUFAs), of omega-3 fatty acids and omega-6 fatty acids are the most crucial molecules required in normal development and functioning of brain and CNS (Schuchardt et al., 2010). These fatty acids are found in brain and retina which are required in adequate amount for the learning process (Tan et al., 2012). The neurocognitive disorders like DD, dyspraxia, ADHD and autism were found to be deficient in these PUFAs (Richardson, 2004; Vancassel et al., 2001; Young and Conquer, 2005). Studies on DD and ADHD provided evidence that inefficiency in fatty acid metabolism may be a factor in the biological predisposition of these disorders (Richardson and Ross, 2000). Proper supplementation of these nutrients may helps in the improvement of the learning abilities (Richardson, 2004).
1.6 Genetic Variations

Molecular genetic markers represent one of the most powerful tools to detect polymorphisms in DNA. Studies on these markers enable the identification of association of genomic variations with heritable traits (Duran et al., 2009). The detection of genetic variation between individuals is a key challenge in current research in genome biology. This variation includes Single Nucleotide Polymorphisms (SNPs), Microsatellites, Insertion/deletion polymorphisms (Indels), Copy Number Variations (CNVs) such as deletions, insertions or duplications, as well as copy number invariant changes like translocations or inversions (Duran et al., 2009; Elbert et al., 2011; da Costa Francez, et al., 2012; Jiang et al., 2013).

Indels

Indels are refers to the mutations that includes both insertions and deletions, are strings of mutated base pairs in DNA and are identified by their genomic position and their length. These are the second most abundant form of human genetic variation and are very frequent in the human genome and present several advantages for population and forensic studies (da Costa Francez, et al., 2012). Most importantly, indels can be easily interpreted, small amplicons, genotyped with simple procedures based on size separation and the possibility of using multiplex PCR. Another advantage is chances of two indel mutation of the same length occurring in the same genomic position are negligible and they can therefore be used to map common ancestry in kinship and human origin studies (Li et al., 2011). Indels of 20 bp or less were established in approximately 24% of Mendelian disease mutations. Half of the Mendelian diseases were linked with the indels
results in the amino acid substitutions (Stenson et al., 2003). The indels present in the coding region may also cause the frame shifts results in the mutant mRNA (Scofield et al., 2007; Hu and Ng, 2012).

**Microsatellite**

Microsatellites are repeats in the sequence of polymorphic DNA loci that vary in length from one to six base pairs. These are the most important markers in genetic characterization. Microsatellites are highly polymorphic, co-dominant, relatively small size and rapidly detectable. Enormous data is available on thousands of microsatellite markers across organisms. Since, these markers are widely accepted for linkage and association studies. These markers also used to identify the genetic relationships between individuals and populations through the estimation of genetic distances. Initially function of microsatellites were unknown, however, later studies have identified that microsatellite play a role in genome evolution (Moxon and Wills, 1999; Duran et al., 2009). These markers are useful in biomedical diagnosis of certain disease linked with the markers. Microsatellite repeat sequences mutate frequently by slippage and proofreading errors during DNA replication that primarily change the number of repeats and thus the length of the repeat string (Eisen, 1999). Because of the difference in length of the alleles, high resolution gel electrophoresis can be used to distinguished, which allows rapid genotyping of the markers in many individuals (Schlo¨tterer, 2000). Microsatellites located in the promoter and exonic region are involved in gene expression and alterations in the function of the gene (Duran et al., 2009). Studies on microsatellite markers of DD showed strongest evidence for association marker located in the first untranslated exon region of the KIAA0319 (Elbert et al., 2011).
CNVs

CNVs are defined as a variable copy number of DNA segments ranging from 50 bp to several megabases. Recently, CNVs have great importance in the study of genetic variations associated with disease phenotypes. These markers ubiquitously distributed in the genome and their importance in the phenotypic diversity and evolution. CNVs involve larger genome region such as changing gene dosage and disrupting genes structure (Jiang et al., 2013). Several studies have recently explored the relationship between these variants and risk of complex disease (Subirana et al., 2011). CNVs within or nearing to the genes affects the expression of genes. Identification of CNVs in both human and model organisms associated with disease phenotypes have confirmed that these structural changes in genome are important in contribution of phenotypic variation (Henrichsen et al., 2009). Studies have implicated CNVs in a range of neurological phenotypes and reported positive correlation of severity of childhood disability with large CNV (Girirajan et al., 2011).

SNPs

SNPs are DNA sequence variations represents single base change occur in 1% of the population. These are the most studied genetic markers among all. The availability of information on variants and human genome sequence facilitated the use of SNPs in biomedical applications. Screening of SNPs in large number of individuals enables the prediction of susceptibility to a wide range of diseases (Duran et al., 2009). Effect of SNPs on the phenotypes is depends on their location. They may appear in coding, non-coding, promoter and intronic region. Exonic SNPs are of two types, synonymous and nonsynonymous. Synonymous SNPs results in formation of same amino acids and non-
synonymous SNPs results in the change of amino acids which might alter the structure and function of the protein. SNPs in promoter, up and downstream region might affect the expression. Identifying the genes and SNPs responsible for the complex disorder is a difficult task because of genetic heterogeneity. Many complex genes and SNPs susceptible to cause the same phenotypes makes the study challenging. Linkage studies have identified many disease related SNPs, confirmed through candidate gene approach. Identification of SNPs through candidate gene approach for a particular disorder requires prior knowledge of disease pathogenesis (Alwi, 2005). Availability of evolving molecular techniques made these markers study easier and possibility of rapid detection.

A promising approach for the study of correlation of specific SNP with particular phenotype of the disease is association analysis. It can be conducted through family based and case-control studies. Case-control studies involve affected and unaffected subjects. The frequencies of the SNPs in the cases were compared with the control group. Application of suitable statistical method defines the significance of the SNP. Family based studies involve both parents and the proband. The frequency of the SNP is compared with the parents, siblings or healthy biological relatives of cases by considering as controls (Spielman et al., 1993; Peltonen et al., 2000).

1.7 Genome - wide linkage studies

There are two ways to identify the genes or the chromosomal region causing a disorder, one is genome-wide scanning and second approach is candidate gene studies. Genome-wide scan is used to identify the susceptible chromosomal region, genes and alleles which can be linked with the disorder. Candidate gene approach is based on prior
studies, which already identified the causal genes of the disorder and replicating these genes in different samples (Hirschhorn and Daly, 2005). Genome wide linkage analysis of DD has identified nine loci (DYX1-DYX9) and several genes located in different chromosomes regions (Williams and O’Donovan, 2006).

DYX1 in Chromosome 15

Linkage study on DD first identified a locus at chromosome 15 with chromosome heteromorphisms in 20% of the families (Smith et al., 1983). Later support for 15q15.1 to 15q21.3 region was obtained in more than five independent populations through linkage analysis of RFLP marker and other microsatellites (Smith et al., 1991; Grigorenko et al., 1997; Schulte-Korne et al., 1998; Chapman et al., 2004; Schumacher et al., 2008). Phenotypic correlation study, showed significant association with reading related measures and spelling. These results gain further support by observing co-segregation of a translocation break point at t(2;15)(q11;q21) with DD in a Finnish family (Nopola-Hemmi et al., 2000). Examination of translocations in four families with DD in Denmark found one of the breakpoints occurred with dyslexia linkage region at 15q21 (DYX1) (Buonincontri et al., 2011).

DYX2 and DYX4 in Chromosome 6

DYX2 is a susceptible locus on chromosome 6p22.3-p21.3 was identified by linkage studies on many microsatellite markers and it is considered as one of the promising locus for DD (Grigorenko et al., 1997, Fisher et al., 1999, Gayan et al., 1999, Grigorenko et al., 2000; Fisher et al., 2002; Kaplan et al., 2002; Grigorenko et al., 2003). Grigorenko et al. (1997) found an association of phonological awareness phenotype to 6p21.3 which was supported by the Gayan et al. (1999) study. Later, same region was
linked with both phonological and orthographic skills (Fisher et al., 1999). Three independent studies have confirmed that 6p21.3 influences various dyslexia-spectrum processes (Grigorenko et al., 2000; Fisher et al., 2002; Kaplan et al., 2002). Genome-wide linkage of microsatellites by sib pair study in 246 dyslexia families of German samples found evidence for a major dyslexia locus on chromosome 6p21. The cognitive trait rapid naming produced significant LOD score (König et al., 2011).

A new locus in the chromosome 6q11.2–q12 named as DYX4 was linked with DD. 6q13-q16.2 was found to be associated with phonological coding dyslexia (Field and Kaplan, 1998). Linkage studies of microsatellites have also identified 6q11–q12 as a susceptible locus for DD in chromosome 6 in Canadian population with phonological coding and spelling (Petryshen et al., 2001).

DYX3 in Chromosome 2

A genome wide study on Norwegian family with 36 dyslexic family members reported the co-segregation three microsatellites on 2p15-p16 with DD (Fagerheim et al., 1999). Linkage analysis in Canadian population using 97 families also provided the evidence for the association of 2p15–p16 region with DD (Petryshen et al., 2000). Later Fisher et al. (2002) also found association of same region with DD. A genome wide study in Finnish sample also confirmed the linkage of 2p11 locus with DD (Kaminen et al., 2003). Recently, a chromosomal translocation at 2p13 (DYX3) found co segregating with DD phenotypes (Buonincontri et al., 2011).

DYX5 in Chromosome 3

Linkage study in a large Finnish family showed chromosome region 3p12–q13 as a susceptible region for DD. A genome wide scan of microsatellites in a large Finnish
family with DD having autosomal dominant pattern of inheritance showed significant linkage between the dyslexia phenotype and chromosomal region 3p12–q13. (Nopola-Hemmi et al., 2001). Microsatellite linkage study in UK and American samples also provided significant evidence for the association of 3p13 with DD (Fisher et al., 2002).

DYX6 Chromosome 18

QTL based genome-wide scans in large in US and UK samples showed a link between a microsatellite on 18p11.2 with single-word reading and phonological processing of DD (Fisher et al., 2002). Another linkage study in UK sample also supported the 18p11.2 association with phoneme-awareness measure (Marlow et al., 2003).

DYX7 in Chromosome 11

The chromosome region 11p15 was reported as one of the locus for DD. This region specifically at 11p15.4 was identified by 7-repeat allele in Canadian population by linkage studies (Hsiung et al., 2004).

DYX8 in Chromosome 1

A targeted linkage study identified association of microsatellite marker between chromosome region 1p34–p36 with DD (Rabin et al., 1993). A finding of translocation between chromosome 1 and 2, 46,XY;t(1;2)(p22;q22) was co-segregating with DD in three family members in a family confirms the previous reports (Froster et al., 1993). A further linkage study on eight families showed a significant association of DD with a wide region on chromosome 1p (Grigorenko et al., 2001). QTL microsatellites linkage analysis was performed on four measures of dyslexia to identify the presence of a DD
gene in 1p34-p36 region in a sample of 100 Canadian families confirmed and strengthened the previous reports by identifying positive results for chromosome region 1p34-p36 with DD (Tzenova et al., 2004). Recently, a translocation break point at 1p36 (DYX8) was observed in four families, where the translocation was co-segregated with the DD phenotype (Buonincontri et al., 2011).

**DYX9 in Chromosome X**

Multipoint QTL analyses of X-linked markers suggested a locus on Xq26 in the UK sample of dyslexia in males than in females (Fisher et al., 2002). Dutch multiplex family with dyslexia also found significance for the chromosome region Xq27 by microsatellite genome linkage analysis (de Kovel et al., 2004). A recent SNP linkage and candidate gene investigation study on 12 French families found significant linkage on Xq27.3 and consequently confirmed the DYX9 region as a robust susceptibility locus (Huc-Chabrolle et al., 2013).

### 1.8 Candidate genes for DD

The break point in the translocation t(2;15; p12;q21) of chromosome 15 identified the first gene of DD namely, *DYX1C1*. Another gene *CYP19A1* found to be located in the same break point region and linked with DD (Nopola-Hemmi et al., 2000; Anthoni et al., 2012). *ROBO1* was also identified through translocation study in a family with DD (Nopola-Hemmi et al., 2001). Linkage studies on microsatellites and SNPs have identified *KIAA0319, DCDC2, TTRAP, THEM2* and *FOXP2* as susceptible candidate genes for DD and several replication studies provided supportive evidence for the
association of these genes with DD (Francks et al., 2004; Deffenbacher et al., 2004; Pinel et al., 2012). Novel DD candidate genes SLIT2, HMGB1 and VAPA were also found associated with DD through linkage analysis (Poelmans et al., 2011).

KIAA0319L located at 1p34.3 having high protein sequence identity to KIAA0319 was linked with DD by identifying SNPs association (Couto et al., 2008). Linkage disequilibrium study in German population showed association of haplotypes of MRPL19 and C2ORF3 with DD (Anthoni et al., 2007). In a study of autism samples DOCK4 in AUTS1 locus on chromosome 7q31.1 was identified as DD candidate gene. Same gene was replicated in sibling study in Dutch samples (Pagnamenta et al., 2010). SNP microarray and Fluorescence In Situ Hybridization (FISH) study identifies a deletion on chromosome region 21q22.3 which harbors four brain-expressed genes, PCNT, DIP2A, S100B, and PRMT2 (Poelmans et al., 2009). In the Finnish sample overlapping haplotypes was found in DGKI associated with DD (Merida et al., 2008). Studies have confirmed the role of DYX1C1, KIAA0319, DCDC2, ROBO1, KIAA0319L, S100B, DOCK4, FMR1, DIP2A, and GTF2I genes in axon guidance (Poelmans et al., 2011).

Three genes MC5R, DYM, and NEDD4L on chromosome 18 were also identified as candidate genes for DD (Scerri et al., 2010a). Few of the genes were linked with the sub phenotypes of DD viz. GRIN2B, LI, CMIP, ATP2C2, and CNTNAP2 were linked with phonological memory (Brkanac et al., 2007; Ludwig et al., 2010; Newbury et al., 2009; Peter et al., 2011) and CMIP is also linked with single word reading and spelling (Newbury et al., 2011; Scerri et al., 2011). These genes are reported as candidate genes of DD but they were not replicated in different samples. Further studies on these
candidate genes will help us better understand the significance and role of these genes in the manifestation of DD (Table 1.1).

DD is a complex genetic disorder; many genes have been involved in the manifestation of DD. Molecular genetics of DD is well studied in different parts of the world and enormous information is available on DD. However, the genetic information of DD in Indian sample is very less. Therefore, investigation of molecular genetic analysis of DD may provide a better understanding of genetic basis of the disorder in Indian population. In view of this, in the present investigation an attempt was made to address the following issues of DD.

OBJECTIVES

1. To identify and establish the mode of inheritance and construction of pedigree of the affected family.

2. To genotype SNPs in strong candidate genes of dyslexia.

3. To correlate phenotypic variations with genetic profile of individuals with DD.

The findings of these objectives were compiled and presented in the following sections of the thesis.
Table 1.1: Approximate locus and candidate genes associated with Developmental Dyslexia.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Chromosome region</th>
<th>Harbored candidate genes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1p34.2</td>
<td>KIAA0319L</td>
<td>Couto et al., 2008</td>
</tr>
<tr>
<td>2.</td>
<td>2p11.1-q11.2, 2p12</td>
<td>MRPL19 &amp; C2ORF3</td>
<td>Anthoni et al., 2007</td>
</tr>
<tr>
<td>3.</td>
<td>3p12</td>
<td>ROBO1</td>
<td>Nopola-Hemmi et al., 2001</td>
</tr>
<tr>
<td>4.</td>
<td>4p15.2</td>
<td>SLIT2</td>
<td>Poelmans et al., 2011</td>
</tr>
<tr>
<td>5.</td>
<td>6p22.1, 6p22.3-p22.2, 6p22.3, 6p22.3-p22.1</td>
<td>DCDC2, KIAA0319L, THEM2, TTRAP</td>
<td>Meng et al., 2005Francks et al., 2004</td>
</tr>
<tr>
<td>6.</td>
<td>7q31.1, 7q32.3-q33, 7q35, 7q31, 7q11.23</td>
<td>DOCK4, DGKI, CNTNAP2, FOXP2, GTF2I</td>
<td>Pagnamenta et al., 2010Merida et al., 2008Peter et al., 2011</td>
</tr>
<tr>
<td>7.</td>
<td>12p12</td>
<td>GRIN2B</td>
<td>Brkanac et al., 2008</td>
</tr>
<tr>
<td>8.</td>
<td>13q12</td>
<td>HMGB1</td>
<td>Poelmans et al., 2011</td>
</tr>
<tr>
<td>9.</td>
<td>14q11.2</td>
<td>LI</td>
<td>Ludwig et al., 2010</td>
</tr>
<tr>
<td>10.</td>
<td>15q21.3, 15q21.1</td>
<td>DYX1C1, CYP19A1</td>
<td>Nopola-Hemmi et al., 2000Anthoni et al., 2012</td>
</tr>
<tr>
<td>11.</td>
<td>16q23, 16q24.1</td>
<td>CMIP, ATP2C2</td>
<td>Newbury et al., 2009Scerri et al., 2011</td>
</tr>
<tr>
<td>12.</td>
<td>18p11.2, 18q21.1, 18q21, 18p11.22</td>
<td>MC5R, DYM, NEDD4L, VAPA</td>
<td>Scerri et al., 2010a</td>
</tr>
<tr>
<td>13.</td>
<td>21q22.3, 21q22.3, 21q22.3, 21q22.3</td>
<td>DIP2A, PCNT, PRMT2, S100B</td>
<td>Poelmans et al., 2009</td>
</tr>
<tr>
<td>14.</td>
<td>Xq27.3</td>
<td>FMR1</td>
<td>Poelmans et al., 2011</td>
</tr>
</tbody>
</table>
Figure 1.1: Activation of brain regions in normals and dyslexics. Inferior frontal gyrus for articulation and word analysis, perietotemporal region for word analysis and occipitotemporal region for word formation (Shaywitz and Shaywitz, 2008).