CHAPTER FOUR

ALZHEIMER’S DISEASE IN ADULTS WITH DOWN SYNDROME

Warren B. Zigman,* Darlynne A. Devenny,* Sharon J. Krinsky-McHale,* Edmund C. Jenkins,†
Tiina K. Urv,‡ Jerzy Wegiel,§ Nicole Schupf,**†† and
Wayne Silverman††,‡‡

Contents
1. Introduction 104
2. Down syndrome/Alzheimer’s Disease Research Program 108
   2.1. Classification and diagnosis 111
   2.2. Adaptive behavior and cognitive processes 115
   2.3. Maladaptive behavior 122
   2.4. Alzheimer’s disease and life span neuropathology
       in individuals with Down syndrome 123
   2.5. Risk factors 125
   2.6. Strategies for future research 133
Acknowledgments 135
References 135

* Department of Psychology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314
† Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314
‡ Mental Retardation & Developmental Disabilities Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20892
§ Department of Developmental Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314
¶ The Taub Institute for Research on Alzheimer’s Disease and the Aging Brain, Columbia University College of Physicians and Surgeons, New York, New York 10032
** Departments of Epidemiology and Psychiatry, Columbia University Medical Center, New York, New York 10032
†† Department of Behavioral Psychology, Kennedy Krieger Institute, Baltimore, Maryland 21205
‡‡ Department of Psychiatry and Behavioral Medicine, Johns Hopkins University Medical School, Baltimore, Maryland 21205

International Review of Research in Mental Retardation, Volume 36 © 2008 Elsevier Inc.
ISSN 0074-7750, DOI: 10.1016/S0074-7750(08)00004-9 All rights reserved.

103
Abstract

Down syndrome is associated with increased mortality rates due to congenital cardiac defects and leukemia early in life, and with Alzheimer’s disease and a tendency toward premature aging later in life. Alzheimer’s disease was once considered an inexorable result of growing old with Down syndrome, but recent data indicate that risk does not reach 100%. Although some individuals exhibit signs and symptoms of Alzheimer’s disease in their 40s, other individuals have reached the age of 70 without developing dementia. This chapter presents a wealth of data from a longstanding longitudinal study with the overall objective of understanding and recounting the mechanisms responsible for these substantial individual differences.

1. Introduction

Down syndrome is the most common chromosomal abnormality leading to intellectual disabilities, and results from any one of three different genotypes. The first, and most common, is free trisomy 21, where three full copies of the 21st chromosome are present (~95% of cases). The second is mosaic Down syndrome (~1% of cases), where some but not all cells are trisomic for chromosome 21. The degree of mosaicism can range from <1% to 99+. The third is translocation Down syndrome, where extra critical regions of chromosome 21 are attached to chromosome 14, 21, or 22 (~4% of cases). Most chromosome 21 translocations are now thought to be isochromosomes where the extra chromosome 21 material originated as a duplication of chromosome 21q material, originating de novo in postzygotic mitosis via misdivision (Gardner & Sutherland, 2004).

The region at the end of the long arm of chromosome 21, including portions of bands 21q22.2 and 21q22.3, represents the “Down syndrome critical region,” the region generally believed to be responsible for much, but not all, of the Down syndrome phenotype. The presence, in triplicate, of the genes within this region is associated with many of the neuropathological and clinical features of Down syndrome (Korenberg et al., 1994; Robakis et al., 1987), although recent results from studies of mouse models suggest a more complex genotype–phenotype relationship (Olson et al., 2007).

In the early twentieth century, mean survival for children with Down syndrome was ~9 years (Penrose, 1949), and during the second half of that century placement of affected individuals in large institutional settings became an accepted practice (Lakin & Stancliffe, 2007). Since then, disability advocacy groups and other proponents of rights for individuals with disabilities have been effective in expanding supports that have led to important improvements in quality of life. However, issues related to aging into late adulthood were largely ignored until quite recently, probably
due to the abbreviated life span that characterized previous generations (Carter & Jancar, 1983). In fact, it was not until 1985 that research which was explicitly focused on aging-related changes in health status and cognition of adults with intellectual disabilities, and in particular those with Down syndrome, began in earnest. The first book focusing on aging of adults with intellectual/developmental disabilities appeared in 1985 (Janicki & Wisniewski, 1985), and since then, a wealth of information regarding the aging process in people with intellectual disabilities has been generated.

One topic, the association between Down syndrome and Alzheimer’s disease, has perhaps received more attention than any other aspect of aging among adults with intellectual/developmental disabilities [including a previous chapter in this series (Zigman, Schupf, Zigman, & Silverman, 1993)]. Presenile dementia in adults with Down syndrome was first recognized more than 130 years ago (Fraser & Mitchell, 1876), and the development of some aspects of neuropathology characteristic of Alzheimer’s disease was noted in several classic studies (Jervis, 1948; Malamud, 1972; Struwe, 1929). Late-onset or sporadic Alzheimer’s disease is the major cause of dementia among older people and currently affects over 26 million people worldwide (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007).

Clinically, Alzheimer’s disease is characterized by a mid- to late-life onset of progressive deterioration in cognitive and functional abilities, with considerable variability in behavioral manifestation. Alzheimer’s disease affects parts of the brain that control thought, memory, and language abilities during its earlier stages and progresses to other areas over time, causing serious declines in an affected individual’s ability to carry out all aspects of daily activities (Victor & Ropper, 2001). Three lesions are particularly characteristic of Alzheimer’s disease: (a) neuritic plaques, extracellular deposits of fibrillar β-amyloid surrounded by degenerating neuronal processes and terminals, (b) intraneuronal neurofibrillary tangles, primarily composed of abnormally hyperphospholated τ-protein, and (c) vascular β-amyloidosis associated with fibrillar amyloid deposition within the vascular wall (Victor & Ropper, 2001). Over time, these pathological processes contribute to synaptic and neuronal loss, deterioration of neuronal networks, brain atrophy, and dementia (Victor & Ropper, 2001).

Alzheimer’s disease has now become a major public health concern due to ever-increasing longevity and the resulting increase in the proportion of the world’s population over the age of 60 [10% in 2005 vs. an estimated 22% in 2050 (World Health Organization, 2005b)]. Annual costs in the United States, both direct (i.e., Medicare and Medicaid) and indirect [i.e., lost productivity of caretakers], have been estimated to exceed $148 billion in 2005, and are projected to increase to over $189 billion by 2015 (Alzheimer’s Association, 2007).

A substantial increase in life expectancy for people with Down syndrome during recent decades can be linked to both societal and medical factors.
Societal attitudes regarding the institutionalization of people with intellectual/developmental disabilities underwent a radical transition beginning in the 1960s, resulting in the depopulation and closing of many of the nation’s institutions that continues to this day (Landesman-Dwyer, 1981). Medical factors included the availability of corrective surgery for congenital cardiac problems (Glasson et al., 2002), common in children with Down syndrome (Yang, Rasmussen, & Friedman, 2002), and all the other general advances in medical care, nutrition and public health practices that have resulted in extensions of life expectancy for all Americans (Silverman, Zigman, Kim, Krinsky-McHale, & Wisniewski, 1998).

Despite improved survival rates, people with Down syndrome continue to experience atypical life span development, with anatomic, immunologic, neurological, endocrine, and metabolic disorders characteristic of the phenotype (van Schrojenstein Lantman-de Valk, Haveman, & Crebolder, 1996). Down syndrome is still associated with increased mortality rates, both during early and later life span development, and age-specific mortality risk remains higher in adults with Down syndrome (Minino, Heron, & Smith, 2006), even compared with other people with intellectual disabilities (Bittles et al., 2002). Whether, and when, life expectancy for people with Down syndrome will equal that of the typically developing population remains an open question, but the outlook continues to improve.

Causes of increased mortality rates early in life are still due primarily to the increased incidence of congenital defects and leukemia. Causes of higher mortality rates later in life may be due to a number of factors, two of which are an increased risk for dementia due to Alzheimer’s disease and an apparent tendency toward premature aging (Yang et al., 2002). Dementia is defined in the current edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1994) as the development of multiple cognitive deficits, involving memory, and aphasia (language impairment), apraxia (motor impairment), agnosia (perceptual impairment), or disturbance in executive functioning. Additionally, dementia is characterized by substantial declines in adaptive abilities and significant functional impairment. Progressive deterioration also occurs in the ability to perform coordinated movements, and affected individuals eventually can no longer walk, show severe signs of disorientation and lose all self-care skills (Reisberg et al., 1986).

Investigators at the New York State Institute for Basic Research in Mental Retardation [now Developmental Disabilities (IBR)] have conducted research on Down syndrome and Alzheimer’s disease during the last four decades. IBR opened its doors in 1966, with Dr. George Jervis as Director. Before his appointment at IBR, Dr. Jervis, a physician, had been a distinguished researcher and the Director of Research at Letchworth Village State School, at that time one of the largest institutions in the world caring for people with intellectual/developmental disabilities. His groundbreaking
studies of the genetic defect responsible for phenylketonuria (PKU) were followed by seminal research regarding the development of Alzheimer’s disease in adults with Down syndrome. He published the first article directly linking Alzheimer’s disease-type neuropathology to clinical dementia in adults with Down syndrome (Jervis, 1948). His interests in the neural underpinnings of behavior led him to develop a brain bank that included over 2,000 cases with their medical and behavioral records. This valuable archive stimulated a wide-range of subsequent research, much of which focused on psychological, neuropathological, and neurobiological aspects of aging and intellectual disabilities (Barcikowska et al., 1989; Popovitch et al., 1990; Silverman et al., 1993; Wegiel, Wisniewski, Dwiewiatkowski, Popovitch, & Tarnawski, 1996), as well as on the linkage between Down syndrome and Alzheimer’s disease (Wisniewski, Dalton, McLachlan, Wen, & Wisniewski, 1985; Wisniewski, Wisniewski, & Wen, 1985).

Dr. Jervis retired as IBR’s Director in 1973 and was succeeded by Dr. Henry Wisniewski, an internationally recognized authority on Alzheimer’s disease, and the association between Down syndrome, atypical aging and Alzheimer’s disease became a major part of IBR’s research agenda. The discrepancy between the ubiquity of Alzheimer’s disease neuropathology in adults with Down syndrome by age 35–40 without apparent signs and symptoms of dementia was one issue of immediate interest (Devenny et al., 1996), as were studies of the prevalence of dementia and Alzheimer’s disease neuropathology in adults with intellectual disabilities without Down syndrome (Barcikowska et al., 1989; Popovitch et al., 1990). The remainder of this chapter will acquaint the reader with an in-depth description of the IBR’s research programs on Down syndrome, aging, and Alzheimer’s disease and the implications of this research for improving the health and well-being of persons with Down syndrome.

In 1993, when a previous chapter regarding Alzheimer’s disease in adults with Down syndrome appeared in this series (Zigman et al., 1993), the discrepancy between the prevalence of Alzheimer’s disease pathology in adults with Down syndrome (i.e., presumed to be 100% by age 40) and the prevalence of dementia (i.e., ~2–5% by age 40 and 70% by age 70) was a major focus. One factor originally proposed to explain the discrepancy between the widespread presence of Alzheimer’s disease neuropathology and the less than universal occurrence of clinical dementia among adults with Down syndrome was diagnostic imprecision caused by preexisting cognitive impairments of varying severity (Silverman et al., 1998). Diagnostic overshadowing, a phenomenon in which signs and symptoms of psychopathology and dementia are attributed to intellectual disabilities rather than a separate disease process (Reiss, Levitan, & Szyszko, 1982) was also considered a very real possibility. However, results of our earlier program findings, as well as those of others, provided convincing evidence that the vast majority of adults with Down syndrome in their 30s and 40s did
not have dementia (Devenny, Hill, Patxot, Silverman, & Wisniewski, 1992; Zigman, Schupf, Urv, Zigman, & Silverman, 2002) and relevant research programs have gone on to address other issues.

Only minor consideration was given in that earlier chapter to explaining the variation in differential risk for dementia evident among adults with Down syndrome, and even less time to theorizing about factors that could reduce risk. Over the last 15 years, the field has advanced, and considerable attention will now be given to factors modifying risk of Alzheimer’s disease/dementia within the population with Down syndrome. Whereas Alzheimer’s disease was once considered an inexorable result of growing old with Down syndrome, recent data indicate that risk does not reach 100%. Although some individuals exhibit signs and symptoms of Alzheimer’s disease in their 40s, other individuals have reached the age of 70 without dementia (Head, Lott, Patterson, Doran, & Haier, 2007; Schupf, 2002; Zigman & Lott, 2007), and it is important to understand the mechanisms responsible for these substantial individual differences if promotion of successful aging is to be maximized.

The remainder of this chapter is divided into eight parts. The first summarizes efforts to develop a test battery that would be sensitive to changes in functional and cognitive ability in adults with Down syndrome. The second describes efforts to develop procedures for classifying signs and symptoms of dementia objectively and reliably, a task complicated by the presence of preexisting cognitive impairments that can vary considerably in their severity within this population. The third reviews findings regarding changes in functional and cognitive capabilities as a consequence of aging and the development of Alzheimer’s disease. The fourth examines the occurrence of maladaptive behaviors and psychiatric symptoms associated with the progression of dementia. The fifth focuses on studies of the influence of neurogenetics and neuropathology on life span development and the Down syndrome phenotype. The sixth examines factors, both genetic and somatic, mediating overall and age-specific risk for dementia. Investigations of biomarkers, physical signs or laboratory measurements that occur in association with the development of a specific disease or disease process (Lesko & Atkinson, 2001) are discussed in the seventh section of this chapter. Finally, the eighth part of this chapter discusses implications of future research to improve the health and well-being of people with Down syndrome.

2. Down syndrome/Alzheimer’s Disease Research Program

Much of the data described below were generated by IBR’s multidisciplinary program focused on aging, and the development of Alzheimer’s disease in adults with Down syndrome funded by the National Institutes of
Health since the mid-1980s. Initially, this program was codirected by Drs. Henry Wisniewski and Wayne Silverman, with Dr. Silverman assuming the role of Principal Investigator in 1997. Early studies included individuals with intellectual disability without Down syndrome, but studies that are more recent have focused solely on adults with Down syndrome, primarily due to their features of atypical aging and dramatically increased risk of Alzheimer’s disease (Zigman et al., 1993; Zigman, Silverman, & Wisniewski, 1996). The common dataset for most of these studies includes descriptions of performance and health status collected during the long-term follow-up, for as much as 15–20 years, of more than 400 adults with Down syndrome over the age of 40 and more than 125 adults with intellectual disabilities without Down syndrome over the age of 60.

The primary requirement for a clinical diagnosis of dementia is evidence of a decline in memory and at least one other aspect of cognition sufficient to impair personal activities of daily living (World Health Organization, 2005a). In the case of Alzheimer’s disease, impairments of memory typically affect the registration, storage, and retrieval of new information during relatively early stages of dementia, but previously learned and familiar material may be lost, particularly as the disease progresses. Evidence of these impairments in the presence of clear consciousness is required to ensure that delirium is not the principal cause. These impairments must have been evident for at least 6 months for a clinical diagnosis of dementia to be made. Further, for a diagnosis of Alzheimer’s disease, alternative causes of dementia must be ruled out (i.e., untreated hypothyroidism, traumatic dementia, Parkinson’s disease, dysfunction associated with late onset depression or other psychopathology, development of sensorimotor impairment, and stroke). In the general population without Down syndrome, the second most frequent cause of dementia is cerebrovascular disease. However, because the presence of Alzheimer’s disease neuropathology is virtually universal in adults with Down syndrome, “pure” vascular dementia would not be an appropriate diagnosis in this population, although there may be some mixed cases (Collacott, Cooper, & Ismail, 1994).

Cognitive impairments of older adults with intellectual disability are, by definition, of longstanding duration, given that onset must have occurred before the age 18 (Luckasson et al., 2002). Therefore, the impact of Alzheimer’s disease, as well as of any other old age-associated dementing disorder, has to be assessed against a background of substantial preexisting impairment (Burt et al., 1998). Currently, there are no broadly adopted protocols for diagnosing Alzheimer’s disease in adults with intellectual disability, largely because tests routinely used to diagnose dementia were never intended to differentiate between dementia and cognitive impairments associated with intellectual disability. In addition, because adults with intellectual disability vary tremendously in cognitive capabilities from person to person, assessment methods need to be developed that can take this
variation in baseline abilities into account. These considerations dictated the structure of the assessment battery. Measures were focused on those that were likely to be sensitive to changes associated with developmental aging and dementia, and/or could be useful in describing profiles of changes that differentiated between these two very different situations. In making selections of procedures, maximizing compatibility with recommendations published by a Working Group focused on assessment of dementia within the population with intellectual disability was desired (Aylward, Burt, Thorpe, Lai, & Dalton, 1997; Burt & Aylward, 2000). Direct participant testing had to be sufficiently brief and had to incorporate frequent breaks to avoid fatigue and to avoid approaching the limits of attention span, both of which could exert strong effects on performance. Given these considerations, the final battery of assessments included measures of adaptive and cognitive functioning together with a comprehensive review of clinical records. This battery included a mix of procedures involving direct assessments of performance and informant reports.

Informant-based assessments included: (a) the Dementia Questionnaire for Mentally Retarded Persons (DMR; Evenhuis, 1992, 1996), a questionnaire measuring changes in social and cognitive functioning suggestive of dementia, (b) Part I of the American Association on Mental Deficiency Adaptive Behavior Scale (ABS) (Nihira, Foster, Shellhaas, & Leland, 1974), an instrument measuring functional abilities, (c) the Reiss Screen for Maladaptive Behavior (Reiss & Valenti-Hein, 1994) to screen for possible depression, psychosis and behavior management problems, the symptoms of which might mimic dementia or be associated with its progression, and (d) a Life Events Questionnaire (LEQ; G. Seltzer, personal communication, January 31, 1997), a measure of stressful life events that might increase risk for dementia or result in a temporary pseudodementia syndrome.

Cognitive abilities of all Program participants have also been described based upon the following individually administered tests: (a) a slightly enhanced version of the Down Syndrome Mental Status Examination (DSMSE) developed by Haxby (1989) and Silverman et al. (2004), (b) the IBR Evaluation of Mental Status (IBREMS; Wisniewski & Hill, 1985), (c) the Test for Severe Impairment (TSI; Albert & Cohen, 1992), (d) an adaptation of the McCarthy (McCarthy, 1972) verbal fluency test (MCVF), (e) the Beery Visual Motor Integration (VMI) test to ascertain construction ability (Beery & Buktenica, 1989), and (f) a modified version of the Selective Reminding Test (SRT; Buschke, 1973; Krinsky-McHale, Devenny, & Silverman, 2002) appropriate for use with the target population. Blood samples have been collected to examine genetic markers that might be associated with risk for Alzheimer’s disease or be sensitive to disease progression, as well as to confirm the clinical diagnosis of Down syndrome for participants without prior cytogenetic diagnoses of Down syndrome. Neurological examinations have also been conducted to determine
differential diagnoses for participants who develop dementia. [Full descriptions of the instrument battery and its measurement characteristics have been previously published (Silverman et al., 2004; Zigman et al., 2004).]

Subsequent to each participant’s full evaluation, conducted at 14–18-month intervals, dementia status was classified in a clinical consensus conference consistent with ICD-10 criteria (World Health Organization, 2005a). Dementia status classifications included: (a) nondemented, indicating that criteria for dementia were definitely not met, (b) questionable, indicating substantial uncertainty regarding dementia status, although some indications of mild functional and cognitive declines were present, (c) possible dementia, indicating that ICD-10 criteria were met but that evidence of progressive decline over an extended period of time was judged to be insufficient for clinical judgment to be “definite,” (d) definite dementia, indicating that ICD-10 criteria were met and there was convincing evidence of progressive decline over time, (e) uncertain with complications, indicating that criteria for dementia were met, but that symptoms might have been caused by some other substantial concern, usually a medical condition unrelated to a dementing disorder (e.g., loss of vision, poorly resolved hip fracture, loss of social support network due to relocation), and (f) undeterminable, indicating that preexisting impairments were so severe that detection of declines indicative of dementia was not possible. Using these case classifications, a number of studies relating diagnostic criteria, patterns of adaptive and cognitive change, risk factors and the development of maladaptive behaviors, and psychiatric symptomology to dementia status, both cross-sectionally and longitudinally, were conducted.

2.1. Classification and diagnosis

As noted above, making a diagnosis of Alzheimer’s disease and/or dementia in adults with preexisting intellectual disability can be a daunting task; in fact, it is significantly more difficult than making the similar diagnosis in typically developing adults. In the general population, there is a baseline level of ability that can be reasonably assumed. While, clearly, there is interindividual variability in intellectual functioning, the vast majority of adults are self-sufficient in daily activities of living as well as in the ability to perform typical cognitive tasks necessary for independent coping within the complex demands of modern society. When these abilities are lost, it is clear that dementia is present, and the true task is to determine its etiology. Adults with Down syndrome, in contrast, have substantial lifelong cognitive impairments of varying degrees that complicate diagnosis of dementia. Clinical presentation can be atypical, both for Alzheimer’s disease and for other conditions causing dementia, and even illnesses completely unrelated to central nervous system function can present as a pseudodementia (either due to secondary symptoms of the condition itself or due to atypical side
effects of medications used to treat the primary concern). Given this situation, the optimal method of diagnosing dementia in adults with Down syndrome is to document substantial decline from previous status. Unfortunately, appropriate baseline premorbid data are unlikely to be available and, even if they are, they may be of uncertain quality/validity.

These issues could be addressed by development of practical dementia assessment methods targeting the population with intellectual disabilities analogous to those currently used in evaluations of elderly adults without intellectual disabilities, but with specific classification criteria anchored to premorbid levels of intellectual ability [e.g., measured against available full scale intelligence quotient test scores (FSIQ)]. When diagnosing dementia in the typically developing population, both informant interviews (e.g., Berg, 1988; Blessed, Tomlinson, & Roth, 1968; Teri et al., 1992) and direct assessments of mental status (e.g., Folstein, Folstein, & McHugh, 1975) can provide valid indications of dementia status from a single administration in most cases, and it would be of enormous value if comparable methods and classification criteria were available for use with adults who have intellectual disabilities. Silverman and colleagues (Silverman, Devenny, Krinsky-McHale, Ryan, & Zigman, 2006; Silverman et al., 2004) have tackled this issue using the DMR, an informant-based measure of dementia status developed by Evenhuis (Evenhuis, 1992, 1996), and the IBREMS, a direct assessment of cognitive status based upon the Mini-Mental State Exam (Folstein, Folstein, & McHugh, 1975), which was modified (Wisniewski & Hill, 1985) to be used with adults who have intellectual disabilities. Silverman (Silverman et al., 2004) evaluated the sensitivity and specificity of these two instruments for classifying dementia status, as determined in the clinical consensus conferences mentioned above. Specificity is defined as the proportion of individuals “without dementia” correctly classified as not having dementia; sensitivity is defined by the proportion of individuals “with dementia” correctly classified.

The DMR generates two scores: the Sum of Cognitive Scores (SCS, reflecting cognitive abilities) and the Sum of Social Scores (SSS, reflecting social skills). By plotting the SCS against FSIQs, Silverman et al. (2004) was able to generate a function that distinguished adults with dementia from those without dementia. As illustrated in Fig. 4.1, classification of SCS falling on or above this curve as positive for dementia produced results that converged well with the Consensus Dementia Status ratings, indicating a strong overall association between SCS and dementia status.

To determine the diagnostic utility of the IBREMS, Silverman plotted total scores (minus colors and concentration scores which for most participants proved to be too easy or too difficult, respectively) against FSIQ and again generated a function that distinguished adults with dementia from those without dementia. Figure 4.2, illustrates this function, and with scores falling below this curve indicating dementia, results were again quite
consistent with the Consensus Dementia Status rating. However, the correspondence between consensus classifications and IBREMS results were imperfect, and, for example, 21% of nondemented individuals were misclassified as having dementia. This rate of false positives would make use in clinical settings impractical, and therefore strategies to improve accuracy were explored.

Silverman evaluated the sensitivity of two combinations of the FSIQ-referenced criteria, one requiring a positive indicator on both the DMR-SCS and the IBREMS to be consistent with dementia and the other requiring a positive indicator on either of the two standards (Silverman et al., 2004).

Figure 4.1 Scatterplot relating the Sum of Cognitive Scores (Evenhuis, 1995) for individuals within each consensus dementia status group. (The plotted line indicates IQ-referenced criteria for distinguishing individuals with dementia from those without dementia.) Reprinted with the kind permission of the American Association on Intellectual and Developmental Disabilities (Silverman et al., 2004).
When both FSIQ-referenced criteria had to be met for a dementia classification to be made, specificity increased to .85, indicating that only 15 of every 100 individuals without dementia (consensus categories of either "nondemented" or "questionable") would be misclassified. In contrast, sensitivity was only .78; indicating that slightly more than 1 in 5 individuals with dementia would be incorrectly classified as not having dementia. An alternative was to classify individuals as having dementia if either one or the other of the two FSIQ-referenced criteria were met. Of course, this is a more liberal criterion and, as expected, specificity dropped to .70, an unacceptable rate of error. In contrast, every case having dementia was now classified correctly, and a way to maintain this high sensitivity while improving specificity needed to be found. Fortunately, this was accomplished by simply adding consideration of caregiver concern into the classification procedure.

**Figure 4.2** Scatterplot relating IQs to performance on the modified IBR Mental Status Examination (IBREMS) for individuals within each consensus dementia status group. (The plotted line indicates IQ-referenced criteria for distinguishing individuals with from those without dementia.) Reprinted with the kind permission of the American Association on Intellectual and Developmental Disabilities (Silverman et al., 2004).
Operationally, if concerns about Alzheimer’s disease or nonspecific memory loss were either present in clinical records or expressed during informant interviews, and either of the two FSIQ-referenced criteria were met, then the case would be classified as having dementia. If neither FSIQ-referenced criterion was met or if no concern about dementia or Alzheimer’s disease were expressed in the clinical record or by an informant, then the case would be classified as not having dementia. This expanded classification rule produced a specificity of .90 and a sensitivity of .89.

The results of these analyses indicated that, in principle, relatively straightforward procedures involving a combination of direct assessments, informant interviews, and an examination of medical records at a single point in time could provide a valid basis for classification of dementia when performance is referenced to an individual’s FSIQ. Because dementia, by its very nature, involves declines in cognition and function, users of these types of assessment tools should prefer to base final diagnoses on actual observations of substantial decline over time, but these findings demonstrated that declines can be inferred even when high-quality baseline descriptions of cognitive capabilities are unavailable. Future efforts should seek additional measures of performance together with criteria that might be more sensitive to early stages of Alzheimer’s disease. It will also be important to confirm the validity of the specific criteria described by Silverman in independent samples of adults with Down syndrome, given that they were generated post hoc (Silverman et al., 2004). Finally, these results can only be generalized to populations with composite FSIQs over 25 and parallel procedures appropriate for use with people having more severe lifelong impairments need to be developed. Once classification criteria conceptually comparable to those described here are in place, more informed decisions regarding diagnosis, prognosis, and evolving treatment options can be made.

The DMR-SCS and the IBREMS are just two of potentially many tools that show promise for classifying the dementia status of adults with intellectual disabilities. When interpreted thoughtfully, these assessment methods should be of clear value to clinicians as they evaluate adults with intellectual disabilities who find themselves in the same position as any other individual with real or suspected dementia.

2.2. Adaptive behavior and cognitive processes

2.2.1. Adaptive behavior

Dementia is defined in the current edition of the Diagnostic and Statistical Manual of Mental Disorders as the development of multiple cognitive deficits, involving memory, and aphasia (language impairment), apraxia (motor impairment), agnosia (perceptual impairment), or disturbance in executive functioning. Additionally, it is characterized by a substantial decline in adaptive abilities and significant functional impairment. Zigman and
The age-associated incidence of significant decline in adaptive behavior, presumed to reflect dementia, and the temporal pattern of decline in specific functional skill domains were examined in a longitudinal study of 646 adults with intellectual disabilities through 88 years of age (Zigman et al., 2002). Cumulative incidence of significant decline in adaptive behavior for adults with Down syndrome increased from less than 4% at age 50 to 67% by age 72, whereas cumulative incidence of significant decline in adaptive behavior for adults with intellectual disabilities without Down syndrome increased from less than 2% at age 50 to 52% at age 88. Rates of dementia in adults with intellectual disabilities without Down syndrome were equivalent to the general population rate of Alzheimer’s disease [i.e., ≈47% affected over age 85 (Evans et al., 1989)]. Given that individual differences in vulnerability to Alzheimer’s disease have been hypothesized to be due to variation in lifelong cognitive capabilities (the cognitive “reserve” hypothesis), adults with intellectual disabilities should be at increased risk. This suggests that factors determining intelligence may have little or no direct relationship to risk for dementia and that other predisposing factors for Alzheimer’s disease may be responsible for the association between risk and lifelong cognitive capabilities within the general population. However, this conclusion needs to be qualified somewhat. Because individuals were recruited varying widely in their age at entry into this study, the possibility that older participants, who should have been at highest risk for dementia, were, as a group, physically more robust than their original birth cohort must be considered. In other words, differential healthy survivor effects may have masked population differences.

Among adults experiencing overall decline on the ABS, Part 1, four separate clusters of adaptive functioning differing in the timing and magnitude of change over time were found (Zigman et al., 2002). Relatively large and early declines in performance were observed for care of clothing/dressing/undressing, domestic activities, and vocational activities. Relatively early, but somewhat smaller declines in performance were seen in responsibility, socialization, economic activities, physical development, travel and general independent functioning activities. Proficiency in these skills may be considered necessary to function competently in everyday activities of daily life outside the home. Clusters reflecting more basic activities of daily living skills declined slightly later. Larger declines were observed for self-direction, toileting, numbers, time and cleanliness. Smaller declines were seen for comprehension, social language, appearance, eating and expression. In general, these results indicated that functional declines
are first noted in skills that are more complex and then progress to more basic and fundamental abilities. Not surprisingly, skills related to eating, understanding spoken language, and ambulating were among the last to be affected. These types of patterns are consistent with the clinical progression of Alzheimer’s disease for adults in the typically developing population (Perneczky et al., 2006).

Three additional studies using two alternative adaptive behavior scales with thousands of participants from both New York and California revealed generally comparable results (Strauss & Zigman, 1996; Zigman et al., 1987; Zigman, Schupf et al., 1996), as has a similar study based in England (Prasher, Chung, & Haque, 1998). As has been stated previously, standard diagnostic methods used to evaluate individuals with suspected dementia in the general population are not appropriate for use with adults with Down syndrome (see Silverman et al., 1998), many of whom have never developed the specific cognitive and adaptive skills that are measured by these assessment instruments. Therefore, the use of the ABS as a surrogate measure of dementia could meet a very real need for informing clinical diagnosis. However, the emphasis of the ABS on functional behavior may result in dementia being diagnosed relatively late in the disease process, as small changes in various higher level cognitive abilities may not yet affect performance measures (Krinsky-McHale et al., 2002). Optimally, a highly sensitive and specific assessment battery will eventually be developed that uses the most reliable and valid aspects of each instrument to classify dementia in Down syndrome at the earliest possible stage.

### 2.2.2. Cognitive processes, aging, and dementia in Down syndrome

#### 2.2.2.1. Program description

Devenny and her colleagues (Devenny et al., 1992; Devenny, Krinsky-McHale, Sersen, & Silverman, 2000; Devenny et al., 1996; Devenny et al., 2005; Devenny, Zimmerli, Kittler, & Krinsky-McHale, 2002; Kittler, Krinsky-McHale, & Devenny, 2004, 2006; Krinsky-McHale, Devenny, Kittler, & Silverman, 2003; Krinsky-McHale et al., 2002) have examined the effects of aging on memory and cognition of adults with intellectual disabilities. The major goals of these studies have been to characterize changes in memory and cognition associated with normal aging in adults with intellectual disabilities and to distinguish them from the declines associated with dementia and to develop methods useful for recognizing Alzheimer’s disease in its earliest stages.

The general research strategy involved recruitment of a large sample of convenience ($N = 192$) and the prospective tracking of status over time, with some individuals followed for almost 20 years. Participants were initially selected on the basis of the following inclusion criteria: no suspicion of dementia by caregivers at the time of their entry into the study, IQ $>35$, age $>30$ years, no uncontrolled seizures or severe motor restrictions, no severe sensory impairments and participation in a community workshop.
Adults were chosen who had overall functioning levels in the mild-moderate range of intellectual disability so that their scores on a cognitive battery assessment could provide reliable and objective measurement of any decline over time that might emerge. (A large number of participants in this study were also enrolled in the multidisciplinary study described above). The test battery included the IBREMS (Wisniewski & Hill, 1985) to evaluate mental status, the modified SRT (Buschke, 1973) to measure episodic memory, the Visual Delayed Match-to-Sample Test (VDMST; Devenny et al., 1992) to measure short-term visual memory, the Digit Span to measure verbal working memory (Wechsler, 1974), the Cued Recall Test (CRT) to measure performance on multiple memory processes, the forward and backward Corsi Span task to measure visuospatial working memory, and the raw scores of the Wechsler Intelligence Scale for Children-Revised (WISC-R) (Wechsler, 1974) to provide a profile of overall cognitive functioning.

Early findings indicated that adults with Down syndrome maintained their overall cognitive abilities at least through their 40s and many maintained them through older ages (Devenny et al., 1996), confirming the findings already described of a disparity between the age of onset of dementia and the presumed onset of the neuropathology of Alzheimer’s disease (e.g., Zigman et al., 1987; Zigman, Schupf et al., 1996). However, decline in cognition associated with both “normal aging” and dementia became evident as participants continued to age.

2.2.2.2. Memory processes Declines in memory are a predominant behavioral indication of early-stage dementia. Memory, however, is not a single entity, but a complex system with multiple components. While it is important to recognize that components of memory can influence one another, and that these interactions may differ in populations with and without intellectual/developmental disability (as well as with and without dementia), selective influences of aging and dementia within the memory system can still be examined.

One component, episodic memory, is involved in the encoding and retrieval of events located in time and place and can be assessed using list-learning tasks. The SRT, a test of episodic memory [modified from a task employed in the general population (Buschke, 1973)], was found to be sensitive to declines associated with normal aging in healthy adults with Down syndrome (Devenny et al., 1996; Krinsky-McHale et al., 2002). In addition, it was also determined that individuals with dementia showed consistent declines on the SRT, and an in-depth evaluation of performance on this task showed that both storage and retrieval of test items were affected (Krinsky-McHale et al., 2002).

While decline in the SRT performance was consistent for adults developing dementia, it has already been noted that, in practice, performance would rarely be assessed before the emergence of concerns about possible dementia.
A test that could distinguish between individuals with and without dementia based upon a single administration would be a more useful clinical tool, and this need seems to be met by a modified version of the CRT (Buschke, 1984). The original version of this test was developed for the general population as another method to assess memory performance, and the method entails presenting a list of items to be recalled together with category cues specific to each item (e.g., ball: toy; mouse: animal). By selecting items of low vocabulary levels, reducing the number of items for presentation and recall, and providing opportunities for initial list-learning, nondemented adults with Down syndrome were able to perform well. More importantly, even individuals with low scores on free recall retrieved many additional items when category cues were provided. In contrast, individuals with early-stage dementia performed poorly, for not only the free recall portion of the test, but also when cues were provided. In a recent analysis including 115 adults with Down syndrome, 32 of whom had a medical diagnosis of Alzheimer’s disease, a total score on the CRT (i.e., free plus cued recall) of \( \leq 23 \) on three trials (maximum possible score = 36) yielded a sensitivity of 91% and specificity of 75% for detecting dementia (Devenny, Krinsky-McHale, & Kittler, 2006 August), suggesting that this method may be useful clinically even with a single administration. Interestingly, this criterion seemed to work well regardless of premorbid IQ, at least within the range tested, suggesting that use of this method in broad practice could be quite straightforward.

To gain a better understanding of aging, dementia, and memory processes, the test battery was expanded to include measures of auditory and visuospatial working memory. Baddeley (2000) has conceptualized working memory as the processes that extract information from the environment (or from longstanding personal knowledge) and temporarily maintain it in consciousness. Baddeley’s framework includes four components, one responsible for controlling processing within the system (Central Executive) and three subsystems responsible for temporary storage of information: (a) the phonological loop for sound and speech, (b) the visuospatial sketchpad for visual information, and (c) an episodic buffer dedicated to forming integrated representations and chronological ordering. The auditory/phonological loop component was examined in one study (Kittler et al., 2004), with a forward and backward Digit span task and by the immediate repetition of word lists consisting of three types of words: monosyllabic, phonologically different words; monosyllabic, phonologically similar words; and multisyllabic words. The visuospatial sketchpad was also studied with a forward and backward Corsi Span task.

Findings replicated the well-established tendency for children and young adults with Down syndrome to exhibit relatively better performance on visuospatial tasks compared to verbal tasks, an advantage that persisted in older adulthood. This contrasted with findings for adults with intellectual/developmental disability from unknown etiologies that showed a more
typical pattern of a relative advantage for auditory working memory. In fact, adults with Down syndrome were poorer than their peers with intellectual/developmental disability from other etiologies on all word lists (Kittler et al., 2004).

Phonological loop processes seem to be preserved in the very earliest stages of dementia, but declines in performance on the tasks became apparent as dementia progressed (Devenny et al., 2000). The presence of verbal intrusions (irrelevant responses made in the course of recalling word lists) suggests difficulty in executive control and might be early indications of cognitive decline. Individuals with Down syndrome who made even a single intrusion on any one of the tasks were more likely to show subsequent declines in other measures of cognition 3 years later (Kittler et al., 2006).

Implicit memory involves a very different type of processing, and reflects the acquisition of knowledge and its retrieval without awareness or intentionality. It is thought to be less sensitive to variations in factors such as cognitive ability, aging, and severity of early to mid-stage Alzheimer’s disease. For example, implicit memory can be measured by a fragmented pictures task (Corwin & Snodgrass, 1987). The procedure requires pictures of familiar objects to be identified from a sequence of segments that starts with a very sparse representation and builds up, through ordered steps, to a completely intact and easily identifiable drawing. The initial task does not require the picture or its fragmented representations to be remembered, but information is “automatically” stored in memory nevertheless, and representation of the images results in picture identification at a more fragmented state in the sequence. This type of implicit memory has been found to be less affected by aging and FSIQ than other aspects of memory (Krinsky-McHale et al., 2003), and in fact it was better preserved in adults with Down syndrome who developed dementia (Krinsky-McHale et al., 2005).

2.2.2.3. Other cognitive processes  Early in the longitudinal studies, it was observed that individuals who were showing signs of early-stage dementia also had difficulty on tasks measuring visuospatial organization, such as the Block Design subtest from the WISC-R (Wechsler, 1974). This task consists of reproducing patterns from models using Kohs blocks in which each block has surfaces that are solid red, solid white, and red and white separated diagonally. However, on this task not all participants were able to achieve a sufficiently high score, even when healthy, to allow for the measurement of change. Therefore, an adaptation described by Haxby (1989) was added that extends the test by eight simpler trials. Participants who were showing signs of memory impairment also experienced significant decline on the combined Block Design task (Devenny et al., 2000). Declines on a paper-and-pencil cancellation task assessing selective attention also occurred as much as 2 years before a diagnosis of dementia in adults.
with Down syndrome (Krinsky-McHale et al., 2005). The task simply requires prechosen target figures embedded in a page of other pictures to be marked, placing minimal demands on memory. Scoring involved consideration of both accuracy and speed. Adults with Down syndrome with what now is suspected to be “mild cognitive impairment” (MCI) were slower and made more errors (crossing out additional test items) than their healthy peers, but they were still able to adequately identify targets. By the time these same individuals reached early-stage dementia, they also had difficulty in selectively attending to targets correctly.

2.2.2.4. Case studies  In addition to the wealth of data provided from the large-scale prospective studies described above, single case studies can also help to illustrate diversity in the outcome of the aging process in adults with Down syndrome. A 70-year-old man with a free trisomy 21 karyotype has been one of the research program’s participants for over 16 years, and his performance profile represents a model of successful aging (Krinsky-McHale et al., 2008). He has mild to moderate intellectual disability and a history of cataracts and hearing loss, normal thyroid functioning, and no congenital heart defect. Across multiple administrations of the assessment battery described above, no signs of substantial declines on measures of memory or adaptive behavior have occurred and visuospatial abilities have shown small declines consistent with normal aging. This case study confirmed that dementia associated with Alzheimer’s disease could be avoided, even through the seventh decade of life, for individuals with Down syndrome despite the presence of a complete third copy of chromosome 21. Additional studies are now needed to identify the specific factors supporting successful aging within this population.

A second case study involved a woman, also with a free trisomy 21 karyotype, who died at the age of 57 after showing cognitive and behavioral declines associated with dementia (Devenny et al., 2005). Descriptions by caregivers indicated lapses in her judgment, social withdrawal, and the presence of an eating disorder 5 years before her diagnosis. At the time of her diagnosis, substantial declines were evident in episodic memory and visuospatial organization. Orientation to time was also quite impaired as measured by the IBREMS. Although caregivers observed that her ability to travel within her immediate neighborhood had clearly declined, she continued to answer questions on the IBREMS related to orientation to place correctly for the next 3 years. This illustrates a pattern of sequential decline in various abilities throughout the progression of dementia and the need for both direct cognitive testing and systematic informant reports. Further examination of declines in memory and other cognitive functions should provide clinicians and researchers with valid and reliable metrics of change as well as objective assessments of cognitive and functional status that
can be diagnostic of both MCI and dementia in adults with Down syndrome throughout the full range of intellectual functioning.

2.3. Maladaptive behavior

In the typically developing population, the development of Alzheimer’s disease is often accompanied by the occurrence of psychopathology and behavior problems (Scarmeas et al., 2007; Teng, Lu, & Cummings, 2007). In fact, disruptive behavior is very common in Alzheimer’s disease and predicts cognitive and functional decline as well as future out-of-home placement (Teng et al., 2007). Research on Alzheimer’s disease in Down syndrome has primarily emphasized adaptive, cognitive, and physiological changes, with psychopathology and maladaptive behavior receiving far less attention. Subtle changes in various maladaptive behaviors, especially among individuals with more severe impairments, may be among the earliest indicators caregivers are likely to notice for individuals who may be in the beginning stages of dementia. Precision in diagnosis is especially important for individuals with Down syndrome where preexisting maladaptive behaviors may be exacerbated by declines in cognitive and adaptive behavior; where any new declines may overtax already limited coping skills; and where individuals with different levels of functioning may present underlying changes in status quite differently.

Midway though the large multidisciplinary study described above, a project was added to investigate behavioral changes (i.e., maladaptive behavior and psychopathology) related to age-associated adaptive declines in adults with intellectual disabilities (both with and without Down syndrome; Urv, Zigman, & Silverman, 2003). Although individuals with no significant adaptive decline displayed stable patterns of maladaptive behavior over a 3-year period, those with declines in functional status showed patterns that were more variable. Certain maladaptive behaviors were related to the onset of adaptive declines, with increasing concerns in some areas emerging even before adaptive declines were noted (e.g., lack of boundaries). Other behaviors increased as adaptive declines developed (e.g., withdrawal). In general, findings again suggested similarities in the course of dementia of adults with and without intellectual disabilities and indicated that increases in selected areas of maladaptive behavior may be early indicators of concern for individuals at risk (Urv et al., 2003).

A second study investigated changes in maladaptive behaviors related to specific stages of dementia in adults with Down syndrome (Urv, Zigman, & Silverman, 2008). Generally, individuals who were neither demented nor affected by MCI exhibited a lesser severity of maladaptive behavior than did other participants. Differences in the patterns of maladaptive behavior in individuals as a function of dementia status were evident in both the breadth of concerns and their severity. Individuals transitioning from “healthy”
aging into what might reflect early indications of Alzheimer’s disease displayed increased aggression, fearfulness, sadness, sleep problems, social inadequacy, stealing, and general regressive behavior. Continued analyses of these data are aimed at determining specific symptoms that can be successfully used as metrics of the onset of MCI and frank dementia.

2.4. Alzheimer’s disease and life span neuropathology in individuals with Down syndrome

Neuropathology characteristic of some key features of Alzheimer’s disease is a virtually universal occurrence in people with Down syndrome over the age of 40 (Mann & Esiri, 1989; Wisniewski, Dalton et al., 1985). In adults with Down syndrome, this pathology is superimposed on a neural substrate already affected by preexisting developmental abnormalities. Therefore, as dementia must be assessed against a baseline of lifelong intellectual disabilities (Aylward et al., 1997), neuropathology must be assessed against neurodevelopmental abnormalities that include defects of neurogenesis, synaptogenesis and lamination producing reductions in the sizes of specific brain structures (Crome, 1972; Raz et al., 1995), and a reduced number of neurons and synapses (Ross, Galaburda, & Kemper, 1984; Wisniewski, 1990).

Neuropathological studies have indicated that although trisomy 21 is associated with prenatal defects of neuro- and synaptogenesis, significant gross brain pathology is not present in utero but emerges during early postnatal development (e.g., Wisniewski, 1990). Brain weight at birth is roughly comparable to (or only slightly lower than) that of normal neonates, but subsequent reduction in maturation results in clear retardation of brain growth by age 5. During the first 5 years of life, significant inhibition of growth of dendritic trees and dendritic atrophy is also observed in children with Down syndrome (Schmidt-Sidor, Wisniewski, Shepard, & Sersen, 1990; Wisniewski, 1990).

In part, the developmental abnormalities and intellectual disability characteristic of Down syndrome might be associated with an extra copy of the MNB/DYRK1A (minibrain-kinase) gene. The gene encoding minibrain-kinase maps to the Down syndrome critical region (HSA21q22.2), and its overexpression has been suggested as a strong candidate for causing cognitive impairments associated with Down syndrome. For example, transgenic mice carrying an extra human minibrain-kinase gene show defects in learning and memory (Smith et al., 1997; Smith & Rubin, 1997). Experimental studies of hippocampal cells overexpressing minibrain-kinase have shown large increases in apoptotic cell death and reduction in neuronal differentiation (Park, Yang, Yoon, & Chung, 2007; Wisniewski, 1990), and this mechanism may underlie the altered neuronal plasticity and intellectual disability observed in Down syndrome (Murakami et al., 2006).
β-amyloid deposition in neuritic plaques and in the wall of brain vessels, neurofibrillary degeneration, and neuronal loss are major neuropathological hallmarks of Alzheimer’s disease. The gene coding for amyloid precursor protein (APP), a protein from which β-amyloid peptide is derived, is located on the proximal/mid part of the long arm of chromosome 21 (21q21.3), and its overexpression is considered a factor critical for the early onset of brain amyloidosis seen in people with Down syndrome. In the early childhood of individuals with and without Down syndrome, the majority of neurons in the cortex and subcortical structures are β-amyloid positive, and the distribution of β-amyloid within the brain established during late childhood is maintained during adulthood. The stable distribution of intracellular β-amyloid without neuronal pathology during essentially the entire life span suggests that β-amyloid within neurons represents a product of normal metabolism. Thus, the extracellular β-amyloid deposited in neuritic plaques contributing to the progression of Alzheimer’s disease would seem to reflect another process (Wegiel et al., 2007).

A unique feature of brain amyloidosis in Down syndrome is the deposition of β-amyloid in diffuse, nonfibrillar, and amorphous plaques. This seems to begin as early as 8 years of age (Leverenz & Raskind, 1998), with many individuals with Down syndrome under age 35 showing these deposits (Kida, Wisniewski, & Wisniewski, 1995). These early plaques appear to have negligible effects on neurons, and their deposition has no observable clinical consequences (Wisniewski & Wegiel, 1995). Thus, there seems to be a period of about 30 years during which numerous but almost exclusively diffuse, nonfibrillized amyloid deposits are formed and this occurs in the absence of any other indication of Alzheimer’s disease neuropathology (Wisniewski & Wegiel, 1995).

The onset of dementia observed in older people with Down syndrome, typically in their 50s, appears to be related to neurofibrillary degeneration and the development of neuritic, fibrillized plaques associated with neuropil degeneration (Sadowski et al., 1999; Wegiel et al., 1996). [The neuropil is a complex network of axonal, dendritic, and glial arborizations that constitute the bulk of the grey matter in which nerve cell bodies are embedded (Stegman, 2006).] While early brain amyloidosis appears to be directly associated with overexpression of the APP gene for people with Down syndrome, the precise mechanisms leading to neurofibrillary degeneration, another key feature of Alzheimer’s neuropathology, is unknown.

Phosphorylation is a process by which a phosphate [PO₄] group is added to a protein molecule. This is a fundamental mechanism controlling the activity of enzymes and receptors, which are switched “on” or “off” by phosphorylation and dephosphorylation, respectively, providing crucial regulation of appropriate cell function. In neurons, phosphorylation is involved in normal neuritic outgrowth and axonal transport processes, and aberrant phosphorylation plays a pivotal role in the accumulation
of neurofibrillary tangles, abnormal twisted protein filaments that form within affected neurons and are composed mainly of hyperphosphorylated \( \tau \)-protein (Hardy & Allsop, 1991).

Recent studies revealed that neurofibrillary tangles are immunoreactive with antibodies detecting minibrain-kinase. A higher prevalence of minibrain kinase-positive neurofibrillary tangles in the brains of people with Down syndrome and Alzheimer’s disease and familial early onset Alzheimer’s disease than in other individuals with sporadic Alzheimer’s disease suggests that overexpressed minibrain-kinase may be the factor modifying the onset and progression of neurofibrillar degeneration in Down syndrome (Wegiel et al., 2008, submitted (in press)). Hyperphosphorylation of \( \tau \)-protein in the brains of transgenic mice with an extra human minibrain-kinase gene also suggests that overexpression of minibrain-kinase could contribute to the early onset of Alzheimer’s disease associated with Down syndrome (Ryoo et al., 2007), and it is important to discover the mechanism by which minibrain-kinase influences \( \tau \)-protein processing and how this mechanism is affected by life span development.

The implications of this body of research extend beyond the origins of Alzheimer’s type neuropathology in adults with Down syndrome. Given that the neurobiological deficits noted in the neural substrate of infants with Down syndrome may develop postnatally, and that the development of neuritic plaques and neurofibrillary pathology tends not to appear until adults with Down syndrome are in their 50s, it is at least theoretically possible that therapeutic inhibition of excessive minibrain-kinase activity may prevent or reduce both developmental abnormalities as well as Alzheimer’s disease-type neuropathology. Clearly, these results should be extended before their implications for intervention are considered, but they do portend hope for preventing or minimizing cognitive impairments associated with the Trisomy 21 genotype.

### 2.5. Risk factors

A major effort has been placed on the discovery of risk factors associated with Alzheimer’s disease within the population of adults with Down syndrome, defined simply as factors that either increase or decrease that risk. The heterogeneity in the clinical expression of Alzheimer’s disease observed within the population of adults with Down syndrome may be due to the additive and/or interactive effects of a number of these risk factors, including, but not limited to, genotypic variation, sex, age, health activity, and diet (e.g., Chace et al., 2007; Patel et al., 2004; Patel, Seltzer, Wu, & Schupf, 2001; Prasher et al., 2008 in press; Schupf, Kapell, Lee, Ottman, & Mayeux, 1994; Schupf et al., 1996, 1998, 2003, 2006, 2007; Schupf, Kapell et al., 2001; Schupf, Patel et al., 2001; Zigman, Jenkins, Tycko, Schupf, & Silverman, 2005; Zigman et al., 2007; Zigman, Schupf, & Silverman, 2005). The search
for factors predictive of dementia in people with Down syndrome closely parallels analogous investigations in the typically developing population. It is important to emphasize that while risk factors are associated with the likelihood that a disease will occur, all individuals at elevated risk will not necessarily develop the disease, nor will individuals at reduced risk be immune (Mausner & Kramer, 1985).

### 2.5.1. Age

One factor that has been conclusively linked to an increase in risk for dementia in Down syndrome is advancing age (e.g., Schupf, 2002; Schupf et al., 1996, 2003; Zigman, Schupf, Haveman, & Silverman, 1997). These studies have consistently found that overall dementia risk increases substantially beginning in the late 40s or early 50s, some 20 years earlier than it does within the general population. However, it is clear that individuals vary considerably in their age at onset. A small minority of adults with Down syndrome begin to experience substantial declines in cognition before age 50, yet another minority is able to mature well into their late 60s or early 70s without experiencing signs or symptoms of Alzheimer’s disease. Thus, there must be risk factors in addition to the presence of Down syndrome that contribute to this heterogeneity (Schupf, 2002).

### 2.5.2. Shared genetic susceptibility

Nondisjunction, or the failure of chromosome pairs to separate properly during meiosis or mitosis, is the principal cause of Down syndrome, and is of maternal origin over 90% of the time (Freeman et al., 2007). Schupf and her associates (Schupf et al., 1994; Schupf, Kapell et al., 2001) therefore postulated that if there was a shared genetic susceptibility for Down syndrome and Alzheimer’s disease, it should only be most evident among mothers and maternal relatives of individuals with Down syndrome. They further hypothesized that evidence of shared susceptibility would be strongest in the population of relatively young mothers for whom having child with Down syndrome at a relatively young age might reflect an accelerated aging process in which they were biologically older than their chronological age. Findings clearly supported these hypotheses. There was a fourfold increase in risk of dementia among mothers who gave birth to their children with Down syndrome under 35 years of age compared with mothers who were older than 35 years of age when their child with Down syndrome was born or compared with mothers of children with other intellectual disabilities. There was no increase in risk of dementia among mothers who were over 35 when their child with Down syndrome was born, and no differential patterns of dementia risk in older versus younger fathers of children with Down syndrome or children with other intellectual disabilities. In a follow-up study of the familial aggregation of Down syndrome and Alzheimer’s disease, Schupf replicated her previous findings.
and clearly determined that the increased risk for Alzheimer’s disease was not reflected in increased risk for other age-related neurological diseases (Schupf, Kapell et al., 2001). This suggests that risk for both Alzheimer’s disease and having a child with Down syndrome at a relatively young age is determined, at least in part, by some common underlying mechanism, perhaps controlling some aspect rate of aging.

2.5.3. Sex differences and estrogen
Evidence of increased risk for Alzheimer’s disease in women compared with men in the typically developing population is reasonably robust (Yip, Brayne, & Matthews, 2006); although see Edland, Rocca, Petersen, Cha, and Kokmen (2002). The most widely accepted explanation of this difference postulates that postmenopausal estrogen deficiency in women contributes to decreased cholinergic function as well as increased β-amloid deposition that over time leads to Alzheimer’s disease (Schupf, 2002). The existing literature regarding sex differences in risk for dementia in adults with Down syndrome is limited. As has been reviewed by Schupf (Lai et al., 1999; Schupf, 2002; Schupf et al., 1998), no firm conclusions regarding the influence of sex can be made at this time. Nevertheless, several lines of evidence support the hypothesis that postmenopausal estrogen deficiency may contribute to individual differences in the cognitive declines associated with Alzheimer’s disease among women with Down syndrome (Schupf, 2002).

In a series of studies, Schupf and her colleagues (Patel et al., 2004, 2001; Schupf et al., 2003, 2006, 1997; Seltzer, Schupf, & Wu, 2001) examined a large sample of women with Down syndrome, in collaboration with the multidisciplinary study described above. Using the identical assessment battery, data were collected every 14–18 months from cognitive assessments, caregiver interviews, medical record reviews, blood assays, and neurological examinations to establish the dementia status of each study participant and to relate performance to measures sensitive to, in this case, estrogen biochemistry.

In one study, women with early onset of menopause (46 years or younger) had earlier onset and increased risk of Alzheimer’s disease compared with women with onset of menopause after 46 years, presumably related to long-term lower estrogen bioavailability for the former group (Schupf et al., 2003). In a follow-up prospective study, postmenopausal women with Down syndrome and lower bioavailable estradiol were more likely to develop Alzheimer’s disease (Schupf et al., 2006). Similar findings were apparent in a series of studies relating estrogen levels to cognitive ability. Premenopausal women performed better than age-matched male peers did while postmenopausal women performed more poorly than age-matched male peers did. Premenopausal women and young men showed no significant declines in cognition over time, while postmenopausal women, but not their matched male peers, showed significant declines in cognitive
function (Patel et al., 2004, 2001). These results support the hypothesis that cognitive declines are associated with estrogen deficiency in older women, and provide convincing support for the hypothesis that reduction in bioavailable estrogen following menopause contributes to the cascade of pathological processes leading to Alzheimer’s disease. In the typically developing population, reductions in bioavailable estrogen levels also have been related either to slower declines in cognitive function and decreased risk of Alzheimer’s disease (Hoskin, Tang, Manly, & Mayeux, 2004), but estrogen or hormone replacement therapy has variously been related either to slower declines in cognitive function and decreased risk of Alzheimer’s disease (Tang et al., 1996) or to increases in risk for Alzheimer’s disease (Shumaker et al., 2004). Clinical trials focused explicitly on women with Down syndrome will be necessary to determine whether estrogen replacement in women with Down syndrome can help to reduce their risk for Alzheimer’s disease and its associated dementia. If initial studies suggest benefits, then additional research when used in the premenopausal period will be needed to determine the most effective treatment regimens.

2.5.4. Cognitive reserve
Down syndrome affects brain development in many ways. These include, but are not limited to, decreased brain weight and size, reduced frontal lobe volume, decreased dendritic branching, and increased apoptosis early in fetal development (Capone, 2001). Typically developing brains can tolerate losses of “small” numbers of neurons, regardless of the causes, and consequences for cognitive and functional capabilities will be negligible until an individual’s capacity to compensate for these losses, referred to as “cognitive reserve,” is exceeded. For the typically developing population, there is support for a cognitive reserve theory of individual differences in risk for dementia (Manly, Schupf, Tang, & Stern, 2005; Riley, Snowdon, Desrosiers, & Markesbery, 2005; Roe, Xiong, Miller, & Morris, 2007; Snowdon et al., 1996; Whalley et al., 2000), with lower literacy or educational attainment related to earlier incidences of Alzheimer’s disease. Because the degree of loss sufficient to exceed an individual’s cognitive reserve should vary as a function of education and premorbid intelligence (Jorm, 1996), it is not unreasonable to assume that people with Down syndrome who function at higher levels [e.g., higher premorbid FSIQs, education, occupation, verbal ability (all of which are highly interrelated)] should be at decreased risk for Alzheimer’s disease compared with their peers with lower levels of functioning. Differences in the design and outcome of the few existing studies regarding cognitive reserve and Alzheimer’s disease risk in adults with Down syndrome make it difficult to draw conclusive inferences. However, in a series of studies where FSIQ was included as a covariate in analyses, FSIQ was not related to risk or age at onset of Alzheimer’s disease for adults with Down syndrome (Schupf et al.,
2006; Zigman et al., 2007; Zigman, Schupf et al., 2005). Although rate of cognitive decline was related to lower initial levels of functioning in a few studies (e.g., Prasher & Chung, 1996), contradictory findings have also been found (Prasher, Chung et al., 1998; Zigman, Schupf et al., 2005). These results, in concert with the previously reported finding that rates of Alzheimer’s disease in populations with intellectual disability without Down syndrome were equivalent to the typically developing population, suggest that factors determining intelligence may have little or no direct relationship to risk for Alzheimer’s disease. These conclusions are clearly intriguing, but it is possible that the older adults with more severe intellectual disability represent the very healthiest segment of their birth cohorts, introducing a “healthy survivor” confound. With the weight of the body of literature supporting the concept of cognitive reserve for the typically developing population, it seems premature to reject the hypothesis at this point, but additional studies critically evaluating the concept should be conducted.

2.5.5. Apolipoprotein E

The Apolipoprotein E (APOE) gene, located on chromosome 19, is the most important genetic risk factor found thus far for late onset Alzheimer’s disease in the typically developing population (Corder et al., 1993; Saunders et al., 1993). The APOE gene, which occurs predominately in three variants or alleles (i.e., $\epsilon_2$, $\epsilon_3$, or $\epsilon_4$), is involved in cholesterol transport and lipid metabolism in plasma (Dupuy et al., 2001) as well as accumulation of $\beta$-amyloid protein in the brains of typically developing elderly people, both with and without Alzheimer’s disease (Lambert et al., 2001; Polvikowski et al., 1995]. In numerous studies, participants with Alzheimer’s disease have been found to have higher frequencies of the APOE $\epsilon_4$ allele compared with those with other APOE genotypes, and those with the $\epsilon_4$ allele have an earlier age of onset of Alzheimer’s disease (Corder et al., 1993; de-Andrade, Larrandaburu, Callegari-Jacques, Gastaldo, & Hutz, 2000; Isbir et al., 2001; Mayeux et al., 1993).

APOE $\epsilon_4$ is also associated with greater deposition of $\beta$-amyloid protein in the brains of adults with and without Down syndrome (Hyman, West, Rebeck, Lai, & Mann, 1995), and as for the typically developing population, increased risk for Alzheimer’s disease has been associated with the presence of an $\epsilon_4$ allele (Deb et al., 2000; Prasher, Chowdhury, Rowe, & Bain, 1997; Schupf et al., 1996). The presence of an APOE $\epsilon_4$ allele has also been related to increased overall risk of mortality for adults with Down syndrome without Alzheimer’s disease (Zigman, Jenkins et al., 2005), and it may be associated with intellectual decline during early adulthood (Del Bo et al., 1997). More positively, the presence of the least common allele, APOE $\epsilon_2$, has been associated with a decreased risk of Alzheimer’s disease for adults with Down syndrome (Lai et al., 1999; Royston et al., 1994; Rubinsztein et al., 1999; Schupf et al., 1996), again showing an effect that parallels observations within
Thus, the influence of APOE genotype on risk for morbidity, mortality, and Alzheimer’s disease in Down syndrome suggests that future studies of Alzheimer’s disease risk in Down syndrome should be mindful of its potential effects (Schupf et al., 2007; Zigman et al., 2007; Zigman, Jenkins et al., 2005).

2.5.6. SORL1

Variants in the sortilin-related receptor gene (SORL1), located on chromosome 11, have been found to increase the risk for late onset Alzheimer’s disease in various populations (Rogaeva et al., 2007). It is thought that SORL1 acts to aid in the disposal of excess β-amyloid protein (Rogaeva et al., 2007). Therefore, the under-expression of SORL1 may contribute to an increase in amyloid β-peptides, leading to the amyloid cascade that characterizes Alzheimer’s disease. Given the high background level of β-amyloid protein in the brains of adults with Down syndrome, it seemed natural that any genetic variation that leads to an increase in amyloid β-protein would significantly affect the risk of Alzheimer’s disease. Lee and colleagues investigated the associations between each of seven variants in the gene for SORL1 to age at onset and risk for Alzheimer’s disease and found that homozygosity for two alleles was associated with a later age at onset and reduced overall risk of Alzheimer’s disease (Lee et al., 2007). These findings indicate a modest association of variants in SORL1 with Alzheimer’s disease within the adult population with Down syndrome. However, any conclusion about the relationship between SORL1 and Alzheimer’s disease in adults with Down syndrome must be considered tentative until further studies clarify the mechanism by which this genotypic variability influences phenotype for this vulnerable population (Lee et al., 2007).

2.5.7. Atypical karyotypes

The Down syndrome genotype encompasses three different patterns of chromosomal abnormalities: free trisomy 21, translocation Down syndrome, and mosaic Down syndrome (previously described). A handful of case studies [reviewed by Schupf (2002)] have been conducted to discern whether atypical karyotypes are associated with better long-term outcomes compared to free trisomy 21. For example, elderly individuals with partial trisomy 21 (i.e., disomy of selected genes) or mosaic Down syndrome may not develop the clinical and/or pathological signs of Alzheimer’s disease at relatively young ages. In fact, variants in genotype have been found in quite elderly people with Down syndrome who remained nondemented, including selective disomy of the APP gene on Chromosome 21 (Prasher, Farrer et al., 1998; Schupf, 2002). Zigman and his colleagues examined the comparative rate of mosaicism in adults with Down syndrome as a function of age and found a significantly increased
rate of mosaicism in adults with Down syndrome aged 65 and older, but the majority of these “old” individuals still had free trisomy 21 (Zigman et al., 2000). With the advent of microarray analyses capable of determining DNA sequence copy number, it seems likely that additional genetic variations will be found to influence risk for Alzheimer’s disease in adults with Down syndrome.

2.5.8. Cholesterol and statins
An interaction among APOE genotype, serum cholesterol level, and Alzheimer’s disease within the general population has been proposed in a number of reports (Evans et al., 2000; Hoshino, Kamino, & Matsumoto, 2002; Isbir et al., 2001; Wehr et al., 2000). Cholesterol is transported by high-density lipoproteins such as APOE, and it has been hypothesized that the relationship between APOE and risk of Alzheimer’s disease may be linked to cholesterol metabolism (Launer, White, Petrovitch, Ross, & Curb, 2001). To date, though, evidence has been inconsistent, with total cholesterol not always significantly related to risk for or severity of Alzheimer’s disease (Evans et al., 2000; Kivipelto et al., 2001; Romas, Tang, Berglund, & Mayeux, 1999). Statins or HMG-CoA [3 hydroxy-3 methylglutaryl-coenzyme A] reductase inhibitors are currently the most widely prescribed class of cholesterol-lowering medication. Statin use has also been found to be related to a lower risk for Alzheimer’s disease in a number of studies (Green, Jayakumar, Benke, & Farrer, 2002; Jick, Zornberg, Jick, Seshadri, & Drachman, 2000), although results of a recent large study were negative (Arvanitakis et al., 2008).

Until recently, no study had examined the relationship between total cholesterol levels, statin use, and Alzheimer’s disease in adults with Down syndrome, but Zigman and his colleagues have found that a total cholesterol level of 200 mg/dl or more was associated with increased risk of developing Alzheimer’s disease in this population (Zigman et al., 2007). Further, for participants with a total cholesterol level of 200 mg/dl or more, statin use significantly lowered dementia risk to a level comparable to that of their peers with lower total cholesterol. If the protective effects of statins can be further validated in a clinical trial, these findings suggest that their use may delay or prevent Alzheimer’s disease onset in vulnerable populations. It must be noted that current clinical trials of statin use for Alzheimer’s disease prevention in typically developing populations have not produced encouraging results (Sparks et al., 2005; Zandi et al., 2005), but the life span neurobiology of adults with Down syndrome and their elevated amyloid levels make this population unique. Therefore, the potential benefit of statin use specifically for individuals with Down syndrome with elevated total cholesterol levels still seems worth investigating.
2.5.9. Biomarkers
Biomarkers are physical signs or laboratory measurements that occur in association with the development of a specific disease or disease process (Lesko & Atkinson, 2001). They can help confirm diagnoses, especially in conditions like Alzheimer’s disease where, with currently available technology, the “gold standard” diagnosis can only be determined by direct examination of brain tissue (rarely from a biopsy, but more usually based upon postmortem findings). Biomarkers can also monitor disease progression, which can be especially useful in quantifying the effects of any available treatment regimen. Because biomarkers are strongly associated with disease risk, detection of early changes in biomarker levels provides an opportunity for early intervention (if effective interventions are available) to delay or prevent disease onset. In Alzheimer’s disease, which is especially difficult to diagnose in adults with Down syndrome, a reliable and valid biomarker would be extremely useful for informing diagnostic decisions, as well as for planning care and treatment.

To date, validated biomarkers for Alzheimer’s disease in adults with Down syndrome have yet to be discovered. However, a few have been investigated. These include measures of the quantity and type of β-amyloid protein found in blood plasma (Schupf et al., 2007; Schupf, Patel et al., 2001), and telomere size in metaphase and interphase preparations as well as on individual chromosomes (Jenkins, Velinov, Ye, Gu, Pang et al., 2006; Jenkins, Velinov, Ye, Gu, Li et al., 2006).

2.5.10. Amyloid β-peptides
As discussed already, Alzheimer’s disease is associated with the deposition of extracellular β-amyloid protein within the brain. Two β-amyloid peptides (i.e., β-amyloid 1–40 and β-amyloid 1–42) found in brain, cerebral spinal fluid, and blood plasma of typically developing adults have been found to be related to cognitive status and incident Alzheimer’s disease (Blennow & Hampel, 2003; Naslund et al., 2000), although conflicting findings can be found (van Oijen, Hofman, Soares, Koudstaal, & Breteler, 2006). β-amyloid 1–40 and β-amyloid 1–42 are generated by sequential proteolytic cleavage by β and δ secretases of the APP gene. In a series of studies using both cross-sectional and prospective analyses, Schupf reported that plasma β-amyloid 1–42 levels but not β-amyloid 1–40 levels in demented adults with Down syndrome were increased compared with nondemented adults with Down syndrome (Schupf, Patel et al., 2001). In addition, participants who were nondemented at baseline with the highest levels of plasma β-amyloid 1–42 levels were more than two times as likely to develop Alzheimer’s disease as those with lower levels (Schupf et al., 2007), and participants with the highest levels of β-amyloid peptide 1–42 were twice as likely to die during the course of the study (Schupf et al., 2007). Although
These findings indicate that progression of Alzheimer’s disease may reflect β-amyloid 1–42 levels in plasma, the precise relationship between brain pathology and systematic β-amyloid metabolism is still unclear. More discouraging with respect to the issue of biomarkers, the overlap in the distributions of plasma β-amyloid 1–42 levels in groups with and without Alzheimer’s disease may reflect that these measures cannot be used to inform diagnostic decisions in clinical settings.

2.5.11. Telomere shortening
Telomeres are sequences of DNA on chromosome ends consisting of a series of repeats of the TTAGGG nucleotide sequence. These DNA sequences undergo shortening with each cell division, serving as markers of a cell’s replicative history and an indicator of cellular aging. Telomere shortening has been linked to Alzheimer’s disease in the typically developing population (Panossian et al., 2003), and Jenkins et al. (2006) hypothesized that a similar association might exist in adults with Down syndrome. Using quantitative telomere protein nucleic acid fluorescent in situ hybridization analyses of metaphase and interphase preparations from age-matched pairs of participants with Down syndrome with and without dementia, Jenkins observed: (a) shorter telomeres in individuals with dementia, (b) that individual chromosomes 1 and 21 could be used alone and/or in combination to detect telomere shortening (Jenkins et al., 2006), (c) that cells from individuals with dementia or MCI had reduced numbers of telomere signals when analyzed using a PNA telomere probe (Jenkins et al., 2007), and (e) shorter telomeres in individuals with MCI (Jenkins et al., 2008 accepted). These preliminary results are encouraging, but the procedure used required samples from each affected case together with his or her matched comparison to be processed simultaneously, and in clinical practice, this circumstance would rarely occur. Additional research is underway to standardize procedures that should eliminate the need for simultaneous processing of paired samples.

2.6. Strategies for future research
Individuals with Down syndrome are at a significantly increased risk for developing Alzheimer’s disease compared to typically developing individuals; this fact has been known for more than 100 years. However, contrary to the generally accepted belief, as recent as 25 years ago, that cognitive decline was inevitable by middle age in this population, there is a more positive outlook for adults with Down syndrome as they age into their senior years.
The studies presented above, as well as others, have demonstrated that successful aging for adults with Down syndrome is possible, even as they age into their late 60s and early 70s (e.g., Krinsky-McHale et al., 2008). It appears that risk of Alzheimer’s disease may be modified by many factors, static and not amenable to change as well as fluid and potentially modifiable through intervention.

The cholesterol findings, for example, seem to indicate that lowering total cholesterol levels and/or using cholesterol-lowering medications such as statins, may lead to a decreased risk of developing Alzheimer’s disease for people with relatively high cholesterol. However, because these findings were based upon an observational study where there was no control over prescription practices or other medical treatments, they are not sufficiently convincing to make any recommendations regarding the broad use of cholesterol reduction practices to reduce lower Alzheimer’s disease risk, and more rigorously controlled clinical trials are needed.

While investments in clinical trials targeting Alzheimer’s disease in the typically developing population have increased substantially in recent years, few studies have included samples of adults with Down syndrome. (Two such trials are currently in progress, but no findings have been published to date.) Whether the lack of clinical trials in adults with Down syndrome is a function of lack of interest or a hesitancy to conduct “greater than minimal risk” studies of individuals without the capacity to provide full informed consent, the scientific community and government agencies must move quickly to encourage research in this area. Given the increasing population of elderly adults with Down syndrome at an elevated risk for Alzheimer’s disease (Silverman et al., 1998), these issues need to be promptly addressed.

Knowledge of biomarkers of Alzheimer’s disease is still at a rudimentary stage. There are some exciting advances in the use of advanced imaging techniques, but such techniques might not be as useful in adults with Down syndrome where the background level of β-amyloid deposition is substantial and neuropathological changes are superimposed on a background of variable abnormal neurodevelopment. Currently, there are no generally available biomarkers with sufficient sensitivity and specificity to predict accurately the onset of dementia or to monitor the rate of Alzheimer’s disease progression.

Advances in molecular biology and neurogenetics may provide the most positive outlook for people with Down syndrome. If further research clarifies the role of specific genes in regulating neurobiological development and brain function throughout the life span, interventions based on this knowledge could prevent or minimize developmental impairments as well as prevent the neurodegeneration associated with Alzheimer’s disease. Of course, these are long-term goals that, today, are more dreams than
expectations. However, it was only a short time ago that survival into adulthood was just a dream for most people with Down syndrome, and there is every reason to believe that current research will provide the foundation for further, perhaps even more impressive, future advances in prevention and treatment.

ACKNOWLEDGMENTS

Supported by NIH grants AG014763, HD35897, HD37425, and HD43960; by funds provided by the National Down Syndrome Society in collaboration with the NIH; by Alzheimer’s Association grants IIRG-07-60558, IIRG-99-1598, and IIRG-96-077; and by New York State through its Office of Mental Retardation and Developmental Disabilities.

REFERENCES


