Protocols to Establish Genotype-Phenotype Correlations in Down Syndrome

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Introduction

Progress in the genetic and physical mapping of chromosome 21 has now reached the point at which it is possible to begin the correlation of the phenotypic components of Down syndrome with imbalance of specific regions of the components of the chromosome. Several preliminary efforts in this direction have already been made and suggest that the phenotypic and molecular analysis of relatively rare individuals with chromosome 21 duplications ("partial trisomy") can be used to specify which regions of chromosome 21 are involved in the generation of specific components of the phenotype (see below). The ultimate goal of correlating genotype with phenotype (phenotypic mapping) is to make it possible to discover which particular genes are responsible for which aspects of the phenotype, thereby permitting the pathogenesis of the

syndrome to be elucidated and, it is hoped, its most serious consequences to be prevented or ameliorated.

To facilitate the process of making a phenotypic map of Down syndrome, a workshop was sponsored by the National Institute of Child Health and Human Development on April 24–25, 1990, to develop protocols for obtaining and recording the necessary phenotypic, cytogenetic, and molecular data. It is anticipated that these protocols will provide the basis for analyzing and comparing the phenotypes and genotypes of individuals with different duplications involving the long arm of chromosome 21. The protocols which have been developed were designed to provide a uniform and precise specification of the phenotype and degree of chromosome imbalance of each individual to be studied. Although they are not intended for use in the diagnosis or investigation of Down syndrome per se, these protocols should nonetheless prove useful for these purposes and possibly for the care of persons with Down syndrome.

Rationale for Making Genotype-Phenotype Correlations from Analysis of Chromosome Duplications

The theoretical basis for phenotypic mapping of components of aneuploid syndromes has already been presented in detail (Epstein 1986, 1990). In brief,

analysis of a wide variety of such syndromes has demonstrated that different aneuploid phenotypes are specific and are distinguishable from one another in so far as overall patterns of features are concerned. From this it has been inferred that the aneuploid phenotypes are, in the main, determined by the specific genes present in the region of imbalance. Although stochastic, environmental, and other genetic factors may influence the phenotype in any individual case, they are not the determinants of the phenotype. Furthermore, comparisons of syndromes resulting from overlapping duplications or deletions or from double aneuploidy (involving two different chromosomes) have shown that it is frequently possible to attribute individual components of a phenotype to imbalance of a particular chromosomal region. These observations, as well as recent work on small-deletion or contiguous-gene syndromes, indicate that individual phenotypic features, particularly those which are quite distinctive, are likely to be due to imbalance of one or a very few genes and should therefore be mappable. These observations also suggest that aneuploid phenotypes resulting from the presence or absence of a whole chromosome are not the result of imbalance of a single gene or of a single narrowly defined critical region. It is likely that a global component of the phenotype, such as mental retardation, will have many genetic determinants, even on a single unbalanced chromosome. However, it is possible that some determinants may have a more powerful influence than others or may determine a specific aspect of the retardation and will, therefore, be susceptible to analysis.

Current Status of Genotype-Phenotype Correlations in Down Syndrome

It is now over 30 years since Down syndrome was found to be caused by trisomy 21, and more than 15 years have elapsed since the role of band q22 in causing the phenotype of Down syndrome was suggested. Two changes in direction have recently been defined. First, it is now clear that genes in other regions contribute significantly to the phenotype. Second, the emergence of the physical map of chromosome 21 has eliminated the uncertainty of cytogenetic analyses and has made possible the molecular definition of regions responsible for specific phenotypic features of Down syndrome. A phenotypic map of Down syndrome based on *cytogenetic* analyses is shown in figure 1. This map, constructed from 17 well-defined cases of chromosome 21 duplications published since 1973, shows the overlaps of duplicated regions which are associated with the feature(s) indicated. The map must, for two reasons, be considered as indicating only the *minimal* regions involved in producing a particular feature. Too little information derives from small duplications, and the data are incomplete with regard to the *lack* of features in many of the patients. Furthermore, such analyses do not indicate the number of genes involved.

The phenotypic map of Down syndrome based on molecular analysis of chromosome 21 duplications is now emerging from studies conducted by a number of groups using the techniques detailed below. There is general consensus that, although the facial features of Down syndrome may be determined by genes in the
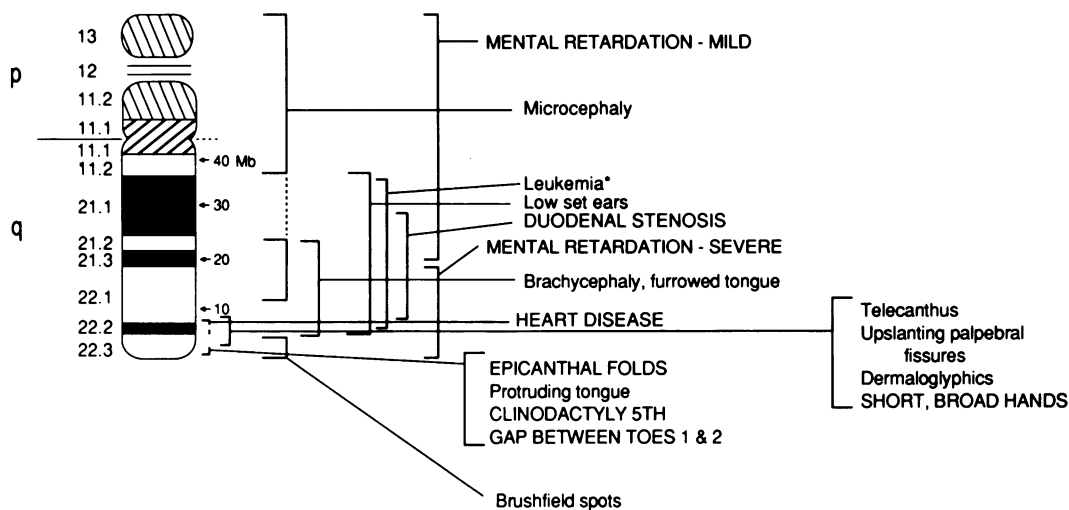

Figure I   Down syndrome phenotypic map based on cytogenetic analysis, 1973–89 (Korenberg 1991)

region of the DNA marker *D21S55*→21qter (Korenberg et al. 1988, 1990; McCormick et al. 1988, 1989; Rahmani et al. 1989), the mental retardation results from imbalance of genes mapping throughout the chromosome. One group has suggested that the region around *D21S55*, between D21S17 and *ETS2*, probably contains genes contributing significantly to the pathogenesis of some of the facial features (flat nasal bridge, macroglossia, folded ears), incurved fifth fingers, gap between first and second toes, hypotonia, and mental retardation (Rahmani et al. 1990). Moreover, the minimal regions likely to contain the gene(s) determining the congenital heart disease and the duodenal stenosis have also been defined, as *D21S55*→21qter (Korenberg et al. 1988, 1990) and *D21S8*→*D21S15* (Korenberg et al. 1989), respectively. Although this clearly is an exciting start, the future goals are equally clear. The size of the regions involved must be reduced through the identification and analysis of further informative cases and the completion of the chromosome 21 physical map. The genes mapping within each region must be identified, and their potential roles in development be assessed. Finally, but of primary importance, each feature of the phenotype must be defined, at the cellular, physiological, physical, and developmental levels. These studies hold the promise of an ultimate understanding of the molecular basis of the components of the Down syndrome phenotype, including mental retardation, congenital heart disease, immune deficits, risk of leukemia, and the link to Alzheimer disease.

Molecular Structure of Chromosome 21

Knowledge of the physical and genetic maps of chromosome 21 underlies the molecular and cytogenetic methods used to determine DNA sequence copy number in rearranged chromosomes 21. Long-range restriction maps of the long arm of chromosome 21 have recently been constructed using somatic cell hybrids, irradiation reduction hybrids, Southern blot hybridization, and pulsed-field gel electrophoresis (PFGE) (Carritt and Litt 1989; Cox et al. 1990; Gardiner et al. 1990). These approaches have resulted in the placement of over 60 unique DNA markers on the long arm, most of which have been ordered. In addition, a genetic linkage map of the chromosome is emerging (Tanzi et al. 1988; Petersen et al. 1991).

At the International Workshop on Chromosome 21 held on April 2–3, 1990, and attended by representatives of most of the laboratories of the world involved

in the mapping of this chromosome, a number of actions relevant to genotype-phenotype mapping of Down syndrome were taken (Cox and Shimizu 1990). A set of 27 ordered, freely available, and well-spaced reference DNA probes spanning the long arm were defined. A set of 12 highly polymorphic genetic anchor markers well spaced along the chromosome were also defined, and a set of five somatic cell hybrids dissecting 21q into well-defined regions were chosen (table 1). These reagents all yield consistent results and are readily available, and it was suggested that kits be prepared for each set of resources. It is now recommended, therefore, that new chromosome 21 arrangements, including translocations, duplications, and deletions, be defined initially in terms of these DNA and genetic markers and hybrids.

Once new chromosome 21 rearrangements are defined in terms of these markers, it will often be possible to refine these boundaries further by using subsets of probes already known to be in the same approximate region as the rearrangement boundary of interest. In general, there is a higher density of well-ordered markers in 21q22→qter, so significantly increased resolution is possible in this region of the chromosome.

Analysis of Data

The workshop participants did not establish a specific approach to the analysis of the clinical, molecular, and cytogenetic data which are expected to be obtained. It is assumed, however, that the data will be handled by methods similar to those that have been used for syndrome identification and classification and for phenotype-karyotype correlations in the past.

There are two principal analytical approaches to the correlation of genotype with phenotype. The first is applicable to features taken separately, not for "patterns," and consists of comparisons of the phenotypes of patients with chromosome duplications, to extract those features that are observed only when specific segments of chromosome 21 are duplicated. The second approach consists of the use of methods of classification to arrange patients with duplications into discrete classes on the basis of their phenotypic relationships and then to look at the chromosomal segments duplicated in such clusters of patients. If clusters so defined are found to correspond to specific duplications, it will be possible to assign a specific phenotype to a chromosome-specific segment.

Several methods of classification developed in the past (Sokal 1974) have been adapted to syndrome definition (Preus and Aymé 1983; Preus et al. 1984).

Table I

Proposed Chromosome 21 Genetic and Physical Markers

Anchor Hybrids	Reference Markers	
	Centromere	
ACEM(q11.1)---		
	D21S16[a]	
	D21S13[a,b]	
	D21S4[a]	
	D21S52[a,b]	
4;21(q11.2)--GM8210		
	D21S1/D21S11[b]	
	D21S18	
1;21(q21.05) ---GM1881		
	D21S8[a,b]	
	APP[a]	
	D21S111[a]	
ACEM(q22.1)---		
	D21S93	
	SOD1[a]	
	D21S58[a]	⌐—*D21S82*[b]
	D21S17[a]	
	D21S55[a,b]	
10;21(q22.2) --|-----------------------------GM9542		
		⌐—*HMG14*[a,b]
	D21S3[a]	
	D21S15[a,b]	
	MX1[a,b]	
	D21S19[a]	⌐—*D21S113*[b]
	CRYA1[a]	
	PFKL[a]	⌐—*D21S112*[b]
	CD18[a]	
	COL6A1[a,b]	
	S100B	
	Telomere	

SOURCE.—Modified from Cox and Shimizu (1990).
[a] STS.
[b] Genetic anchor marker.

Experience has shown that *both the analysis of individual features and the classification of patients into homogenous classes (syndromes) by the methods of numerical taxonomy require the development of a list of characters or descriptors showing a high interobserver consistency*. It is to this end that the clinical protocols that follow have been developed. Phenotypic characters may be coded in a binary manner (present/absent), as counterstates (hyperplastic/hypoplastic), or as ordered multistates arranged according to severity or quantitative classification. For the assessment of specific phenotypes related to different duplications, the similarity of each pair of subjects is scored with respect to each character. A similarity matrix based on all possible pattern combinations found in all individuals is constructed, and a total similarity coefficient is calculated for each arrangement of two subjects. This matrix is then analyzed by a combination of clustering or ordination (principal component analysis) techniques to search for identifiable partial duplication syndromes.

The Protocols

The protocols for specifying the cytogenetic and molecular status of each individual and for recording his or her clinical status are presented in the following sections of this report. As much as has been possible, the clinical protocols have been designed so that the

items may be checked off or filled in, and these sections should be largely self-explanatory. However, because of the complexity of the evaluation of central nervous system function, an Appendix describing the rationale for the proposed neurological and psychological evaluations has been prepared. The detailed evaluation of the neurological (including neurophysiological and anatomical) and psychological aspects of chromosome 21 duplications is considered to be of particular importance because of the evidence that particular areas of cognitive functioning and language may be particularly impaired in Down syndrome (see Appendix).

Protocols for the Molecular, Cytogenetic, and Clinical Analysis of Persons with Chromosome 21 Duplications

Molecular Analysis of Chromosome 21 Duplications

The molecular methods for determining DNA sequence copy number are of two types: those based on quantitative densitometry, with or without the analysis of restriction-fragment polymorphisms, and those based on direct estimation of radioactive signals. Both types of methods estimate the copy number of DNA sequences in aneuploid DNAs by comparing the signal from a sequence of unknown copy number with that of a reference sequence the copy number of which in the aneuploid DNA is known. The ratio of these signals in the aneuploid DNA is then compared with the corresponding ratio derived from diploid DNA. All methods are labor intensive and exacting, but they are capable of generating clear results when the appropriate variables are carefully controlled.

Southern blot dosage analysis. — This technique allows the assessment of copy number of any unique chromosome 21 sequence. Southern blots are constructed with restriction enzyme–digested aneuploid and diploid DNAs, hybridized simultaneously with both a probe for a potentially duplicated region of chromosome 21 and a reference probe, and the resulting band signals are measured by densitometry. This process is repeated with a series of chromosome 21 probes until the full extent of the duplicated and nonduplicated regions is defined (see above and table 1).

It is important to note that a variety of technical considerations, including the detailed aspects of gel running, transfer, fixation, and hybridization, all significantly affect the outcome (protocols available from J.R.K. on request). Because of this, criteria for technical acceptance of an autoradiogram should be developed prior to viewing results from a given blot.

Reference sequences may be located on the same chromosome (which controls for chromosome loss) or on different chromosomes (which obviates problems with complex rearrangements) and, for best results, should be hybridized simultaneously with the unknown copy-number sequences and should be of similar band size and shape to avoid problems of differential DNA loss between hybridizations. Standardized methods that are known to result in complete enzyme digestions must be used. Multiple lanes of both aneuploid and diploid control DNAs should be run, preferably in an alternating pattern, and the diploid DNAs used for the control should be isolated from the same tissue as the patient sample. Exposures should be carried out to generate bands for analysis that are in the linear range of the film, and statistical analysis should be performed on the results. The standard errors of the resulting ratios determine whether three copies of a given sequence may be differentiated from two copies. It is obviously easier to reach statistical significance for 2:1 copy assessments (as in deletions) than for 3:2 copy assessments (in duplications).

Although exacting, this technique is broadly applicable to the analysis of all DNA sequences and does not require family members to be studied. A further advantage of both this and the method utilizing RFLPs (see below) is that sources of background signal are clearly seen and, in most cases, are separable.

Densitometry. — Densitometric analysis is used to compare radioactive signal intensities obtained by autoradiographic exposures of Southern or slot blots. It is essential that signal intensities are within the linear-response range of both the film and the densitometer being used. The linear range of the film can be determined by exposing the film to radioactivity, X-rays, or white light. In the first case, Whatman 3-mm filter strips can be spotted with ^{32}P-labeled DNA in 1.5-fold increments (Tanzi et al. 1987). In the second method, a U.S. National Bureau of Standards Penetrometer (wedge step gradient) is used in conjunction with X-ray exposure of the film to determine linearity directly in a stepwise fashion. In the third case, the film is flashed with white light at a series of time intervals to determine the linear range. In all of these techniques, the resulting density signals produced on the film are measured after exposure and development, and the values are plotted against the respective exposure modality (amount or duration). The linear range of the film is that in which a constant change in exposure generates a constant change in density when plotted on a log-linear scale. above and below this range,

a given exposure will result in a density change smaller than expected.

In the experimental situation, the absolute density of a band or slot is compared with the linear-range density values determined by the scale constructed. Multiple exposures may be required to bring blot or slot bands to the appropriate range. When weak signals are expected, the film may be preflashed to increase the density uniformly to close to the linear range. Generally, the linear range of most films is short and lies in a limited region of 1–2 OD units. There is a second linear range with a shallow slope in the region below 1 OD. However, it is important to note that different brands and types of film will have different linear-response profiles. Moreover, densitometers, even those of the same brand and model type, also vary in the ranges at which they remain linear.

Analysis of RFLPs. — This method allows the determination of the copy number of polymorphic sequences. The Southern blot method described above may be combined with RFLP analyses to determine DNA sequence copy number. In this case, the existence of three parental alleles is assessed either by the presence of a third distinct band or by the increased copy number of one band relative to a second as judged by densitometry (Tanzi et al. 1987). This technique is the simplest when parental alleles are available for analysis and highly variable multiallelic systems which can distinguish each parental allele have been defined. However, there are few such systems currently available. Therefore, in assessing duplications in which any two of the three alleles present are identical, the analysis requires densitometry and comparison of the ratio of autoradiographic bands in the aneuploid DNA to the normal reference ratio determined for that particular allelic system as seen in the parent of origin. Consequently, the densitometric considerations detailed above still apply, although the analysis is made somewhat easier because the distinction reduces from 3:2 to 2:1. In the absence of parental DNA samples, ratios may be compared with DNAs from normals in the population known to carry the same polymorphism. PCR technology will be particularly useful for this method when sequenced polymorphisms are available for closely spaced alleles. A considerable number of DNA polymorphisms resulting from short sequence repeats are being developed and used in such studies.

Slot blot methods. — Designed as an alternative to the other two methods, the slot blot method also allows the assessment of copy number of any unique chromosome 21 sequence (Blouin et al. 1990). The principle

of the method is as follows: DNAs from the person with the chromosome 21 duplication, from a known individual with trisomy 21 (the trisomic control), and from a diploid control are loaded on the same membrane at a series of concentrations by using a slot blotting apparatus. Successive hybridizations with chromosome 21 probes and with a non–chromosome 21 reference probe are then performed, and the resulting signals are quantitated by densitometry. The signals for the reference probe are plotted against those for the chromosome 21 probe for each DNA preparation, and the straight line is calculated by linear-regression analysis. A statistically significant difference between the regression line for the trisomic control and that for the normal subject must be observed. The regression line from the patient with the chromosome 21 duplication is then tested statistically to determine whether it matches with data from either the trisomic control or the normal control (a copy of the gene-dosage program is available from P.M.S. on request). Absence of hybridization background is checked on a slot without DNA and by systematically running control Southern blots. The observation of a significant difference between the trisomic and normal controls suggests that repetitive sequences or other artifacts may not seriously interfere with the estimate of gene copy number. Repeat experiments should give the same results. Attention to using undegraded high-molecular-weight DNA and to complete access of the filter to the DNA (no bubbles) is critical to success. Linearity of signal density must also be considered in this technique.

Direct counting of hybridization signals may be used to evaluate the radioactive signals from both Southern blots and slot blots in all methods described. This has been validated both for the slot blot method utilizing a machine to directly evaluate slot signals (Chettouh et al. 1990) and for methods involving standard scintillation counting (Korenberg et al. 1989). The advantage of this approach is that, although the technical artifacts of Southern blot technology remain, the complexities of densitometry are avoided. However, as yet the methods are not broadly available.

Other potential approaches. — The use of PFGE technology for quantitative Southern blot analysis is not recommended, and its application to the routine definition of chromosome breakpoints is not presently an efficient approach. This is because, despite significant effort, only three breakpoints have been unambiguously detected in over 20 that were examined. Moreover, PFGE is not yet a routine laboratory procedure. Finally, restriction-site polymorphisms, probably due

to differential methylation, may complicate the use of PFGE to search for breakpoints.

An approach which offers some potential advantages for analysis of chromosome 21 rearrangements is the capture of rearranged chromosome 21's in Chinese hamster ovary (CHO) somatic cell hybrids by published procedures (Moore et al. 1977). Use of the Ade-C (GART-deficient) CHO mutant has proved almost invariably successful here. These mutants require purines for growth, and the GART gene, which allows growth in the absence of purines, is located in the distal region of band 21q22.1. Molecular probes for the GART gene and flanking regions are now available, so it should be possible to predict with some confidence the outcome of this approach in any given case. Rearrangements involving other chromosomes in translocations often offer additional advantages, since at least one member of the translocation pair can be captured. Selectable markers often exist on the non–chromosome 21 member of the pair, giving additional opportunities to selectively retain rearrangements of interest.

The advantage of capturing a rearranged chromosome in a hybrid is that whether a particular DNA sequence is present becomes an all-or-none result, rather than a 2:1 or 3:2 dosage effect. Disadvantages include the required cytogenetic analysis of hybrids, the rearrangements that occur in human chromosomes maintained in hybrids, the limited number of selective systems, and the requirement for selective media in which to create and maintain hybrids. The choice of whether to construct a hybrid would depend on whether the rearranged chromosome contained the selectable gene and on the resources and skills available to the investigator carrying out the analysis.

Cytogenetic Analysis of Chromosome 21 Duplications

Banding techniques. — Optimum chromosome evaluation of the patient with Down syndrome and other than classic trisomy 21 may require the use of several different cytogenetic techniques and approaches. When a translocation has been identified or when there is an intrachromosomal aberration, high-resolution banding at the 700–850-band level is needed for breakpoint analysis. The method most frequently used to achieve this band level involves synchronization with methotrexate, release of the block, and harvest at a time calculated to yield the highest number of early-metaphase/prophase chromosomes. When even higher band levels are necessary, i.e., to approach the

1,000–2,000-band level illustrated by Yunis (1981), additional procedures are done.

The use of two banding techniques, each giving complementary information, is recommended for breakpoint determination. The overall most crisp and precise technique for banding chromosomes at the 600–850-band level is trypsin G-banding. This technique best reveals the fine gray bands found at high resolution levels in most pale-staining regions, such as at the ends of most chromosomes. Fluorescent R-banding done with the Latt dual stain method, as modified by Schweizer (1980), is the next choice because there are sharper demarcations between bright and dull regions than occur with other fluorescent techniques. Further, it provides R-bands as a contrast to the trypsin G-bands and produces differential brightness among those R-bands that are the G-band-pale regions of many chromosomes. Finally, this technique is singularly effective for the production of high-resolution chromosome bands after in situ hybridization with fluorescent probes described below. Giemsa R-banding after synchronization and BrdU incorporation is also widely used (Viegas-Pequignot and Dutrillaux 1978).

In situ hybridization. — When a subtle chromosome 21 abnormality is suspected but is not confirmed or is unclear with the use of the above banding techniques, in situ hybridization with chromosome 21–specific probes may be used to clarify the chromosomal status. The choice of the particular probe to be used is critical, for, to be informative, the sequence detected by the probe must be within the rearranged segment.

The traditional in situ hybridization technique using tritrium-labeled probes permits the probe sequence to be short (as small as 0.4–0.5 kb), but it requires 5 d or more of exposure in the dark, examination of 50–200 cells, and either construction of an idiogram or statistical analysis for significance. Also, the exact site of hybridization on the chromosome is not evident, because of grain scatter, emulsion thickness, and other factors.

The newer nonradioactive techniques, such as biotinylation of probes which are then detected with fluorescent avidin, generally require a much longer probe sequence (2–3 kb). However, these methods allow precise localization to both chromatids of the chromosomes bearing a homologous sequence. Thus, a more exact localization is possible. Examination of fewer cells is needed for significance, and the process can be completed overnight (Lawrence 1990).

The new in situ hybridization technique of "chromosome painting" may also prove quite useful, especially initially, to identify abnormalities of specifically suspected chromosomes that are not otherwise readily evident. In this technique, a chromosome 21–specific DNA library is amplified, biotinylated, prehybridized with competitor DNA to remove repeated sequences, and then hybridized in a manner similar to that used for unique-sequence probes. If the library is complete, the entire chromosome 21, excluding the regions of highly repeated sequences, will "light up," as will any part of chromosome 21 which may have been translocated to a different chromosome. A similar probe which generates an R-banding pattern may be constructed using PCR technology with primers from the Alu sequence (Baldini and Ward 1991).

Down syndrome with apparently normal karyotype. — Evaluation of the patient with the phenotypic features of Down syndrome but with an apparently normal karyotype requires additional considerations. At least two explanations are possible: undetected mosaicism may be present, or an extremely subtle chromosome 21 translocation/rearrangement may be present.

Mosaicism for standard trisomy 21 in blood may be statistically excluded by the examination of 20 ($P = .05$) and 100 ($P = .01$) cells. However, in this situa-

tion, a greater number of cells (200–300) is frequently examined. When the test is negative, further analysis of mosaicism in fibroblasts may be warranted by the clinical evidence for Down syndrome.

Undetected chromosome 21 material located elsewhere in the karyotype may be found, as indicated above under "chromosome painting," for relatively large regions. However, smaller regions may be detected with the use of chromosome 21 region-specific DNA sequence mixtures that generate a more intense hybridization signal. Even finer distinction of duplicated regions may be assessed by using either in situ hybridization with well-defined cosmids or gene dosage with unique probes.

Clinical Analysis of Chromosome 21 Duplications

The protocol for recording the phenotype of a person with a chromosome 21 duplication is presented in tabular form suitable for direct reproduction and use. Specific references are provided for the items pertaining to dermatoglyphics and to the nervous system. General reviews containing relevant references for other items in the protocol may be found in the work of Pueschel and Steinberg (1980), Pueschel and Rynders (1982), Epstein (1986), McCoy and Epstein (1987), and Trisomy 21 (Down Syndrome) (1990).

PROTOCOL FOR RECORDING PHENOTYPE OF PERSON WITH CHROMOSOME 21 DUPLICATION

Identifying Information

Date: _____ month/day/year

Name: _____ _____
    Last   First

Date of birth: _____ month/day/year

Address: _____

Ethnic group: _____

Place(s) of birth of parents: _____

Telephone number: _____

Pregnancy & Delivery

Was the pregnancy abnormal?   Yes _____ No _____ Unknown _____

If yes, was there:
  Bleeding or spotting _____
  Maternal illness (specify) _____
  Maternal skin eruptions _____
  Excess weight gain _____
  Alcohol _____
  Drugs _____
  Toxemia _____
  Eclampsia _____
  Other (specify) _____

Length of gestation _____ weeks
Birth weight _____ kg
Birth length _____ cm
Head circumference at birth _____ cm
Mother's age at birth _____ years
Father's age at birth _____ years

Apgar scores   1 min. _____ 5 min. _____ 10 min. _____

Was the delivery or postnatal course abnormal?   Yes _____ No _____ Unknown _____

If yes, was there:
  Excessive length of labor (hours) _____
  Fetal distress _____
  Need for respiratory support _____
  Neonatal seizures (specify type, duration, therapy) _____
  Sepsis _____
  Intracranial hemorrhage _____
  Other (specify) _____

Family History

Is there a family history of:

Miscarriage(s)	Yes ___	No ___	Unknown ___	If yes, how many? ___
Abortion(s)	Yes ___	No ___	Unknown ___	If yes, how many? ___
Stillbirth(s)	Yes ___	No ___	Unknown ___	If yes, how many? ___
Birth defects in siblings	Yes ___	No ___	Unknown ___	If yes, how many? ___

If yes, please describe:

Date that possibility of Down syndrome was first suspected. _____ Age: _____
month/day/year

Pedigree:

Photographs of individual at birth and intervals thereafter should be obtained.

Clinical Information

Age of subject: _____ years _____ months.

Does subject have family resemblance? Yes _____ No _____ Unknown _____

Measurements

Height	_____ cm	Percentile on DS growth chart (Cronk et al. 1988)	_____ %
Weight	_____ kg	Internipple distance	_____ cm
Head circumference	_____ cm	Penis length	_____ cm
Inner canthal distance	_____ cm	Testicular volume	_____ ml
Palpebral fissure length	_____ cm	Hand length	_____ cm
Ear length	_____ cm	Palm length	_____ cm
Chest circumference	_____ cm	Tanner stage	_____

	Yes	Yes, but parent(s) &/or sib-ling(s) also	Doubtful	No	No, but too young or too old	Unknown	Comments
Head							
Brachycephaly							
Open sagittal suture							
Third fontanel							
Flat face							
Other abnormalities/anomalies							
Neck							
Broad, short neck							
Loose skin of neck							
Webbing							
Other abnormalities/anomalies							
Eyes							
Upward slant of palpebral fissures							
Epicanthal fold(s)							
Obstructed lacrimal duct(s)							
Blepharitis							
Brushfield spots							
Peripheral iris hypoplasia							
Nystagmus							
Strabismus							
Congenital cataract							
Acquired cataract							
Keratoconus							
Myopia							
Hyperopia							
Other abnormalities/anomalies							

217

	Yes	Yes, but parent(s) &/or sib-ling(s) also	Doubtful	No	No, but too young or too old	Unknown	Comments
Nose							
Flat nasal bridge							
Anteverted nostrils							
Other abnormalities/anomalies							
Mouth							
Down-turned corners							
Prominent lower lip							
Mouth kept open frequently							
Protruding tongue							
Furrowed tongue							
Narrow, short palate							
Cleft uvula/palate							
Submucous cleft							
Other abnormalities/anomalies							
Teeth							
Oligodontia							
Malformed teeth							
Malerupted teeth							
Abnormal spacing							
Microdontia							
Periodontal disease							
Other abnormalities/anomalies							
Ears							
Prominent upper helix							
Adherent lobuli							
Helix root crossing concha							
Narrow ear canals							
Abnormal hearing test (see Neurological)							
Other abnormalities/anomalies							
Heart							
Atrioventricular canal							
Ventricular septal defect							
Atrial septal defect							
Persistent ductus arteriosus							
Mitral valve prolapse							
Tetralogy of Fallot							
Other abnormalities/anomalies							

218

	Yes	Yes, but parent(s) &/or sibling(s) also	Doubtful	No	No, but too young or too old	Unknown	Comments
Gastrointestinal Tract							
Tracheo-esophageal fistula							
Duodenal stenosis/atresia							
Annular pancreas							
Imperforate anus							
Hirschsprung disease							
Celiac disease							
Umbilical hernia							
Diastasis recti							
Pyloric stenosis							
Other abnormalities/anomalies							
Musculoskeletal							
Hip dislocation							
Patella subluxation							
Atlantoaxial/atlantooccipital instability							
Hyperextensibility of joints							
Other abnormalities/anomalies							
Skin and Hair							
Alopecia							
Vitiligo							
Keratosis							
Early greying							
Other abnormalities/anomalies							
Hands and Feet							
Brachyclinodactyly							
Single fifth finger crease							
Single transverse palmar crease							
Sydney line							
Increased gap: 1st and 2nd toes							
Webbing between 2nd and 3rd toes							
Other abnormalities/anomalies							
Other							
Hypothyroidism							
Other thyroid disorder							
Diabetes							
Psychiatric disorder							

219

	Yes	Yes, but parent(s) &/or sibling(s) also	Doubtful	No	No, but too young or too old	Unknown	Comments
Behavior disorder	___	___	___	___	___	___	
Seizures	___*	___	___	___	___	___	*Type _____
Frequent infections	___	___	___	___	___	___	Frequency _____
Leukemia	___	___	___	___	___	___	

Are there other anomalies or abnormalities that have not been mentioned?   Yes _____   No _____   Unknown _____

If yes, specify:

Onset of menarche _____ years
Onset of menopause _____ years
Age of death _____ years
Cause of death

Onset of symptoms of Alzheimer disease _____ years
Onset of significant intellectual decline _____ years

Autopsy findings:

220

Laboratory Studies

<u>Hematological</u>

RBC count _____   Hematocrit _____ %   Differential
WBC count _____   Lymphocytes _____ %   PMNs _____ %
Platelet count _____   Size _____
MCV _____   MCH _____   MCHC _____
Fetal hemoglobin _____ %   Hemoglobin _____ g/dl
Erythropoietin (if anemic/plethoric) _____
Leukemoid reaction or transient leukemia: Yes _____ No _____   Cyanotic heart disease Yes _____ No _____
                                                            If yes: Age of onset: _____
                                                            Highest WBC: _____
Platelet serotonin _____   Age of normalization of WBC: _____

Leukemia: ALL _____   AML _____   AMKL _____   Other _____   M# _____
What was response to therapy? _____   Present Status? _____
Karyotype of leukemia cells: _____
Bone marrow morphology:
     Fibrosis: Yes _____   No _____
     Megakaryocytic and erythroblastic markers by cytofluorometry: _____

<u>Immunological</u>

Serum levels: IgG _____   IgA _____   IgM _____   IgG _____
     IgG subclasses:
CD4 + _____ %   CD8+ _____ %   Ratio _____
TCR-γδ+ cells _____   CD16 + NK cells _____
IL-2 production by antigen (bacterial, viral)-stimulated lymphocytes _____   Control (essential) _____

If thymus is available, formalin fixed paraffin blocks should be prepared for morphologic studies
     Morphology (Hassall corpuscle size and structure, cortex/medulla - ratio and nature of junction):

[Fresh frozen tissue blocks should be prepared for eventual immunohistochemistry for MHC class II (HLA DR) and ICAM-I expression
and in situ hybridization for cytokine gene expression studies.]

<u>Endocrine</u>

TSH _____   FSH _____
T3 _____   LH _____
T4 _____
IGF-1 _____
IGF-2 _____
Thyroid autoantibodies _____
Bone age at <15 years _____ yrs at _____ yrs

<u>Other</u>

Whole blood serotonin _____
Serum thromboxane level _____
PGE$_2$ _____

Dermatoglyphic Analysis

Enter pattern codes from table below

	Left	Right
I. Hallucal area	___	___
II. Great toe	___	___
III. Palmar interdigital area-1	___	___
IV. Palmar interdigital area-2	___	___
V. Palmar interdigital area-3	___	___
VI. Palmar interdigital area-4	___	___
VII. Palmar triradius	___	___

	Left	Right
VIII. Hypothenar area	___	___
IX. Simian crease	___	___
X. Flexion crease on 5th finger	___	___
XI. Digital pattern-1 (thumb)	___	___
XII. Digital pattern-2	___	___
XIII. Digital pattern-3	___	___
XIV. Digital pattern-4	___	___
XV. Digital pattern-5	___	___

Hopkins Index (calculated according to Table 3 of Bolling et al. 1971) _____

Pattern codes (Borgaonkar et al. 1971)

I Hallucal area
0 - Tibial arch
1 - Distal loop - ridge count ≤20
2 - Distal loop - ridge count >20
3 - Whorl
4 - Tibial loop
5 - Whorl with seam
6 - Tented arch
7 - Fibular arch
8 - Fibular loop
9 - Proximal arch

II Great toe
0 - Arch
1 - Tibial loop
2 - Fibular loop
3 - Whorl
4 - Tented arch

III-VI Palmar interdigital areas (1 to 4)
0 - Absence of pattern
1 - Distal loop
2 - Whorl
3 - Arch
4 - Vestigial pattern
5 - Whorl with seam
6 - Carpal loop

VII Palmar triradius
0 - Absence of t
1 - t
2 - t'
3 - t"

VIII Hyothenar area
0 - Absence of pattern
1 - Ulnar loop
2 - Radial loop
3 - Carpal loop
4 - Whorl
5 - Vestigial pattern
6 - Distal loop
7 - Arch
8 - S pattern
9 - Tented arch

IX Simian crease
0 - Absent
1 - Present
2 - Doubtful

X Flexion crease 5th finger
0 - 2 Creases
1 - 1 Crease

XI-XV Digital patterns (digits 1 to 5)
0 - Arch
1 - Ulnar loop
2 - Radial loop
3 - Whorl
4 - Whorl with seam
5 - Tented arch
6 - Vestigial loop
7 - Vestigial whorl
8 - Ulnar loop w/ central pocket
9 - Radial loop w/ central pocket

222

Neurological and Psychological Information

Clinical Neurology

<u>Birth through 4 months</u>

State of infant[a]
Time since last feeding _____ hrs.

	Normal/present	Abnormal/absent
Muscle tone	___	___
Spontaneous body movements	___	___
Blink reflex	___	___
Rooting response	___	___
Suck	___	___
Auditory response	___	___
Palmar/plantar grasp	___	___
Patellar jerk	___	___
Ankle clonus	___	___
Plantar responses	___	___
Traction response	___	___
Ventral suspension	___	___
Withdrawal	___	___
Automatic stepping	___	___
Placing reaction	___	___
Moro response	___	___

<u>5 months through 6 years</u>

	Normal/present	Abnormal/absent
Standard neurological assessment: If abnormal, specify how:	___	___
Atlantoaxial/atlantooccipital instability[b]	___	___

<u>7 years through 18 years</u>

	Normal/present	Abnormal/absent
Standard neurological assessment: If abnormal, specify how:	___	___
Atlantoaxial/atlantooccipital instability[b]	___	___
Behavior[c]	___	___

<u>≥19 years</u>

	Normal/present	Abnormal/absent
Standard neurological assessment If abnormal, specify how:	___	___
Atlantoaxial/atlantooccipital instability[b]	___	___
Behavior[c]	___	___
Pre-Alzheimer disease assessment[d]	___	___

223

Neuropsychology and Language

<u>Birth through 4 months</u>: no evaluation

<u>5 months through 6 years</u>

Hearing assessment[e]   Normal ____   Abnormal: Conductive ____   Sensorineural ____   Mixed ____
                                                      Mild ____   Moderate ____   Severe ____

Measures of general cognitive development
For function < 30 months of age level
  Bayley Scales of Infant Development[f]   Mental age ____
  Ordinal Scales of Psychological Development[g]   Level attained ____

For function ≥ 30 months of age level
  Kaufman Assessment Battery for Children[h]   Mental processing composite age equivalent ____
  Peabody Picture Vocabulary Test - Revised[i]   Age equivalent ____

Measures of adaptive behavior
  Vineland Adaptive Behavior Scale[j]   Composite age equiv. ____   Communication age equiv. ____   Motor age equiv. ____

Language structure
  Mean length of utterances[k]   MLU ____
  Reynell Developmental Language Scales[l]   Expressive age equiv. ____   Age equivalent ____
  Temple University Short Syntax Inventory[m]   Age equivalent ____   Comprehension age equiv. ____

<u>7 years through 18 years</u>

Measures of general cognitive development
  Kaufman Assessment Battery for Children[h]   Mental processing composite age equivalent ____
  Peabody Picture Vocabulary Test - Revised[i]   Age equivalent ____
  Vineland Adaptive Behavior Scale[j]   Composite age equiv. ____   Communication age equiv. ____   Motor age equiv. ____
  Wechsler Intelligence Scale for Children - Revised[n]

Language structure
  Mean length of utterances[k]   MLU ____
  Patterned Elicitation Syntax Test[p]   Age equivalent ____
  Test for Auditory Comprehension of Language - Revised[q]   Age equivalent ____
  Test of Language Development[o]

Phonology

Arizona Test for Articulation[s]

Shriberg/Kwiatkowski intelligibility measure[t]

Age equivalent _____	% intelligibility _____	
% consonants correct _____	% intelligibility _____	

Memory function[u]

Verbal memory — Word span _____ — Age equivalent _____

Non-verbal memory — Object span _____ — Age equivalent _____

≥ 19 years

All tests recommended for 7 through 18 years

Assessment for dementia[v] _____

Neurophysiology

All Ages

Auditory Brain Stem Evoked Responses[w]

Central condition times at 60 dBHL (in z scores)

Latency wave I _____

Latency wave V _____

I-V interval _____

I-III interval _____

III-V interval _____

I-II interval _____

III-IV interval _____

IV-V interval _____

Latency-Intensity & Amplitude-Intensity Functions (in z scores)

	Wave V	
dBHL	Latency	Amplitude
0	_____	_____
5	_____	_____
10	_____	_____
15	_____	_____
20	_____	_____
40	_____	_____
60	_____	_____
80	_____	_____

Latency of wave V at 20 dBHL minus that at 60 dBHL (in z scores) _____

Click Threshold: weakest level at which wave V is reliably present (in dBHL) _____

<u>≤7 years</u>

Event Related Potentials[x] (in z scores)

<u>Novel Stimuli</u>
Nc   P3a

	Nc	P3a	
Latency: auditory	——	——	N_C at F_z
visual	——	——	P3a at C_z
Amplitude: auditory	——	——	N_C at F_z
visual	——	——	P3a at C_z
Scalp topography: auditory	——	——	F_z/P_z ratio
visual	——	——	

<u>≥7 years</u>

Event Related Potentials[y] (in z scores)

<u>Target Stimuli</u>   <u>Novel Stimuli</u>
Nc   P3b   Nc   P3a

	Nc	P3b	Nc	P3a	
Latency: auditory	——	——	——	——	N_C at F_z
visual	——	——	——	——	P3a at C_z
					P3b at P_z
Amplitude: auditory	——	——	——	——	N_C at F_z
visual	——	——	——	——	P3a at C_z
					P3b at P_z
Scalp topography: auditory	——	——	——	——	F_z/P_z ratio
visual	——	——	——	——	

Neuroimaging   (Repeat all tests yearly once dementia is suspected in persons >40 years old)

<u>All ages</u>

Quantitative MRI scan
Lobar and gyral dimensions
Ventricular volumes ——————   CSF volume ——————
Whole brain volume ——————

<u>≥19 years</u>

PET scan ("resting state" glucose metabolism)[z] ——————

Neuropathological Examination

<u>All ages</u>

Brain weight   ___ g

			Biparietal diameter ___ cm
Frontal-occipital diameter	___ cm		
Malformation of superior temporal gyrus	Yes	No	
Size in relation to cerebral hemisphere (ratio)	Cerebellum ___	Brainstem ___	

Microscopic
    Is there a decrease in number of small neurons in cerebral cortex
    (by quantitative comparison with control)?   Yes   No ___

<u>≥ 19 years</u>

Neurofibrillary tangles and senile plaques[aa]	Yes ___	No ___
Premature age-related myelin pallor[bb]	Yes ___	No ___
Ventricular dilatation	Yes ___	No ___

a   Prechtl (1977)

b   Evaluation for the presence of atlanto-axial dislocation: History - neck/back pain, difficulty walking, weakness in arms, acquired urinary incontinence. Examination findings - limitation of neck motion, weakness in arms/legs, diminished biceps, triceps, brachioradialis reflexes, increased tendon jerks at knee and ankle, clonus, plantar extensor responses, sensory level. Laboratory studies - radiographs of neck in extension/flexion, possible CT or MRI of cervical spine (if clinically indicated), somatosensory evoked responses in the legs (if clinically indicated).

c   Screening test for neurobehavioral disorders including psychosis, compulsive behaviors, stereotyped mannerisms, self injurious behaviors, and attention deficit disorder (see Behavioral Checklist, Achenbach and Edelbrock 1983).

d   Pre-Alzheimer disease assessment: History - change in personality (loss of motivation, increased irritability), decline in activities of daily living skills, increasing awkwardness of gait, onset of a new seizure disorder (type/length/medication), urinary/fecal incontinence.

e   To be classified as normal hearing, most studies require a cutoff of 25 dBHL ISO for 500, 1000, 2000, 4000, and 6000 Hz bilaterally. All speech and language testing should be carried out by a speech pathologist.

f   Bayley (1969)

g   Uzgiris and Hunt (1975)

h   Kaufman and Kaufman (1983)

i   Dunn and Dunn (1981)

j   Interview edition, survey form (Sparrow et al. 1984)

k   Brown (1973). Age norms and details on coding are provided in Miller (1981)

l   Reynell (1977)

m   Gerber and Goehl (1984)

n   For child ≥ 12.5 years approaching normal intelligence (Wechsler 1974)

o   Newcomer and Hammill (1988) - when appropriate in individuals with near normal language

p   Young and Perachio (1983)

q   Carrow-Woolfolk (1985)

r   The Illinois Test of Psycholinguistic Abilities (Kirk et al. 1968). Two subtests should be administered to children 7 years and above. The express reason for including this test is to replicate the robust finding of relatively advanced motor expression in DS children compared to overall performance and compared to other mentally retarded individuals matched on age and IQ (Bilovsky and Share, 1965; McCarthy, 1965). The subtests to be included are "Grammatic Closure" (a test of the ability to complete sentences with words that have the correct syntactic markers) and "Manual Expression" (the ability to express concepts with manual gestures).

s   Fudala and Reynolds (1986)

t   Shriberg and Kwiatkowski (1982)

u   Verbal memory subtests from the Kaufman battery including digit span and memory for objects. This should be supplemented with a word span measure following Varnhagen et al. (1987). Nonverbal memory subtests from the Kaufman battery involving memory for handshape. This shoud be supplemented by a block tapping span from Corsi (1972).

v   Neuropsychological battery from Haxby (1989) designed to assess dementia in Down syndrome: tests of praxis, the ability to create longterm memories, recall of personal information and temporal orientation, visuo-spatial construction, and visuoperceptual discrimination.

w   Widen et al. (1987)

x   Event related potential (ERP) testing to novel stimuli: visual and auditory analogs of Karrer and Ackles (1988).

y   From 7 years on, use ERP testing to target and novel stimuli: Courchesne et al. (1977); Lincoln et al. (1985). Subjects press a button in response to target stimuli only.

z   Baseline PET scan should be considered between 19 and 35 years. Repeat PET scan should be considered at > 35 years if there are findings of dementia.

aa This requires samples from hippocampus, amygdala, and association cortices with special staining for senile plaques and neurofibrillary tangles.

bb Premature age-related myelin pallor is best seen in the occipital pole - particularly in the corona radiata.

Acknowledgments

Appendix

Development of Protocols for Neurological and Psychological Evaluations of Persons with Down Syndrome

The clinical neuroscience of Down syndrome may be best viewed as a series of developmental sequences, the manifestations of which are age dependent. For this reason, the clinical protocols have been organized into epochs which have special significance for the development and senescence of the individual with Down syndrome. The following age categories have been observed in protocol development:

I.   Birth–4 mo
II. 5 mo–6 years
III. 7–18 years
IV. 19–35 years
V.   Greater than 35 years

Although any such partition of development is arbitrary, these categories mark many of the neurological milestones seen in Down syndrome. In the neonatal period (I), the presenting neurological reflexes are characteristic of the disorder. In infancy and early childhood (II), language development seems to present specific problems for the child with Down syndrome, and there is a deceleration in the rate of head-circumference growth. During the midchildhood years (III), there are further consolidations of language and cognitive development, and the clinical problem of atlantoaxial/atlantooccipital instability may appear. In the remaining epochs (IV and V), the neurobiological observations pertaining to accelerated loss of function

and to Alzheimer disease require a specific series of measurements and observations.

The purpose of the clinical neuroscience data base is to acquire phenotypic information about the nervous system of the individual with Down syndrome, information which may ultimately correlate both with clinical measures in other systems and with genotypic finding. Because of the complexity of many of these measures, we have chosen to categorize them according to the following disciplines: clinical neurology, neuropsychology and language, neurophysiology, neuroimaging, and neuropathology.

Clinical Neurology

The developing nervous system has a limited number of recognizable clinical manifestations of disease, and these are often not specific for etiology. For this reason, there is no pathognomonic neurological presentation for Down syndrome, although several features occur with regularity. One of these is generalized hypotonia, which is present at birth and persists throughout early childhood. The etiology of this muscular hypotonia remains unknown. In addition to hypotonia, Cowie (1970) has noted delayed dissolution of early reflexes and automatisms—specifically, grasp reflexes, Moro response, and automatic stepping. Abnormal or deficient responses have included the traction response, position in ventral suspension, and patellar jerk. These findings are reported to correlate well with developmental performance at age 10 mo. A consequence of ligamentous laxity is an atlantoaxial/atlantooccipital instability (Pueschel et al. 1987) which poses risk to the cervical spine of perhaps 15%–20% of children with Down syndrome (Pueschel and Scola 1987), although symptomatic manifestations are reported to be rare.

As the child with Down syndrome matures to an adult, another series of neurological concerns arise in regard to Alzheimer disease and precocious aging (Lott 1982). First described by Jervis (1948), it has been established that virtually all brains from individuals with Down syndrome over age 40 years show the neuropathological features of Alzheimer disease but that a considerably smaller percentage actually develop clinical symptoms of the disorder (Oliver and Holland 1986). When symptomatic, individuals with Down syndrome and Alzheimer disease appear to show a degenerative syndrome beginning with personality change, loss of daily living skills, and apraxias with progression to a neurovegetative state over months to years. The clinical problem of Alzheimer

disease in Down syndrome has received much attention and has been approached from each of the disciplines in the protocol.

Neuropsychology and Language

Over and above the more cognitive impairments, specific deficits in linguistic structure (both syntax and phonology) and in auditory verbal memory occur in Down syndrome (Burack et al. 1988; Fowler 1988, 1990). Unfortunately, however, these areas are not typically represented on omnibus IQ tests. The battery of measures outlined in the protocol includes a general assessment of cognitive functioning at each age, as well as the means to construct characteristic patterns. The goal with each subject is to determine whether there is a disparity between (a) the mental age functioning on general IQ measures and (b) age-equivalent scores on syntax, phonology, or memory. To assure reliable assessment of individual constructs (IQ, memory, syntax, phonology), three separate measures are recommended for each. The suggested measures were selected as being especially analytic, as having valid age norms, and for clarity and availability. For languages other than English, speech pathologists should select measures which assess the areas of interest in a similarly analytic fashion. In addition, it is critical to assess hearing (both pure tone and impedance), structural defects affecting phonology (e.g., cleft palate), and visual acuity, before interpreting the results of language and cognitive testing. A questionnaire is helpful in documenting variables which may influence cognitive and language measures—e.g., variables such as history of chronic ear infections, participation in early-intervention programs, parental education levels, and time spent in institutionalized settings. In general, the use of multiple measures allows for convergence of findings, which tends to make the results more meaningful than does a single measure alone.

The *measures of general cognitive assessment* will provide an IQ score, a level of cognitive functioning, and a mental age equivalent. The latter variable is the most helpful, since it can be compared with performance on specific measures of linguistic structure, intelligibility, and memory. When justified by the child's level of functioning, the examiner should move up to the next level of testing. For each age epoch, three measures of general cognitive functioning are recommended:

1. The first measure is a well-normed omnibus measure of general intelligence which incorporates motor, performance, and verbal factors and which is rela-

tively free of acquired knowledge. The *Bayley Scales of Infant Development* (Bayley 1969) is recommended for the youngest children and has been widely used in Down syndrome research. For children functioning at the preschool level and beyond, the *Kaufman Assessment Battery for Children* (K-ABC; Kaufman and Kaufman 1983) is well standardized for both normal and exceptional children. The *Wecshler Intelligence Scales for Children—Revised* (Wechsler 1974) should be utilized only with higher-functioning older individuals.

2. As a second measure of general intelligence, the battery includes a separate measure dedicated to receptive vocabulary, often used as the core variable in general intelligence measures such as the Stanford-Binet. The Peabody Picture Vocabulary Test—Revised (PPVT-R; Dunn and Dunn 1981) serves this purpose well; the PPVT-R has been standardized on the normally developing 30 mo–adult population.

3. The third measure recommended is the *Vineland Adaptive Behavior Scales: Interview Edition, Survey Form* (Sparrow et al. 1984). This measure of adaptive functioning is appropriate from infancy to adulthood and has been extensively used in individuals with Down syndrome.

These measures provide verbal and nonverbal subscores, as well as scores on subtests specific to memory. In Down syndrome, the findings show that measures of IQ, general cognitive functioning, and mental age are at least 2 SD below the norm and that performance on these global anchor measures exceeds performance on measures of language, phonology, and memory. All three tests provide norms for both English- and Spanish-speaking children; the PPVT-R and Vineland tests could be adapted to other languages, allowing comparison of absolute scores.

In individuals with Down syndrome, the development of *linguistic structure* is impaired not only relative to general cognitive development but also relative to other "verbal" abilities, including communicative function (Beeghly et al. 1990) and vocabulary knowledge (Evans 1977; Miller 1988). This deficit in language structure is apparent in the length and complexity of sentences generated spontaneously, as well as in the structure of sentences elicited under more controlled conditions. In the early stages of language development, a particularly useful index of overall language level is the *mean length of utterances* (MLU) produced by the child (Brown 1973; Miller 1981). This measure is widely used with normally developing children and in special populations as well (Scarbor-

ough et al., in press). It is important to complement an MLU measure with an elicitation task and with a measure of sentence comprehension. In children functioning below the normal 3-year-old's language level, the *Reynell Developmental Language Scales* (1969) assess comprehension and some elicited production; the *Temple University Short Syntax Inventory* (1984) provides a brief check on sentence-imitation skills. At more advanced language levels, these needs can best be met with the *The Patterned Elicitation Syntax Test* (1983) and the *Test of Auditory Comprehension of Language — Revised* (Carrow-Woolfolk 1985). The latter test is relatively free of a lexical confound and has proved sensitive to individual differences in language function in Down syndrome (Sommers and Starkey 1977).

Phonological development is a notable area of difficulty in Down syndrome (Crosley and Dowling 1989). A standardized articulation test, the *Arizona Test of Articulation* (Fudala and Reynolds 1986), measures the adequacy of the subject's production of segments in isolated contexts and can be scored for language age and percent of intelligibility. To complement this, we recommend a measure of intelligibility of running speech (Shriberg and Kwiatkowski 1982; Shriberg 1986).

Auditory verbal memory has long been noted as a major psycholinguistic deficit in Down syndrome (Varnhagen et al. 1987). This deficit is maintained for both visual and verbally presented material which must be held in verbal store. In addition, memory is of some interest because older demented individuals with Down syndrome have diminished abilities to form new long-term memories, compared with individuals with Down syndrome of the same age who are not demented (Haxby 1989). To closely examine the process of cognitive change in older individuals with Down syndrome, we recommend the battery used by Haxby for groups IV and V.

Neurophysiological Measures

Neurophysiological testing using event- related potential (ERP) techniques should be performed on all age groups. Testing will fall into two domains: recordings of auditory brain stem–evoked responses (ABERs) and recordings of auditory and visual ERPs associated with novelty and attention. These testing domains have been of particular interest in studies of Down syndrome.

In regard to ABER assessment, neural responses I–V will be recorded (Wilden et al. 1987). The interpeak

intervals of responses I-II and III-IV have been noted to be relatively reduced, and the interpeak interval of IV-V has been noted to be prolonged (Squires et al. 1986). The latency-intensity function of wave V is typically steeper than normal in subjects with Down syndrome, especially in those with high-frequency (8,000 Hz) hearing loss (Squires et al. 1986; Widen et al. 1987), and wave V response amplitude is also reduced (Widen et al. 1987).

Novelty-and-attention-related ERP responses (e.g., P3b, Nc, P3A) will be obtained as per Lincoln et al. (1985) and Courchesne (1977) for Down syndrome age groups III-V and in a fashion analogous to Karrer and Ackles (1988) for Down syndrome age groups I and II. Auditory ERP responses will be elicited by target phonemes and novel sounds; the visual ERP protocol will be analogous to the auditory procedure. For all novelty-and-attention ERP responses (e.g., P3a and P3b), peak *latencies* are longer than normal in subjects with Down syndrome (Lincoln et al. 1985; Muir et al. 1988). Also, ERP response *amplitudes* (e.g., P3b) tend to be smaller than normal in Down syndrome age groups III-V (Lincoln et al. 1985; Muir et al. 1988). In addition, the *scalp topography* of the attention-related P3b response is abnormal in Down syndrome (Lincoln et al. 1985). These abnormalities may be more exaggerated in aged and demented subjects with Down syndrome (Muir et al. 1988).

Neuroimaging

The neuroimaging protocols follow in part from the neuropathological data concerning brains of persons with Down syndrome (see below) and from quantitative computed tomography (CT) studies of adults with Down syndrome with or without dementia. The most accessible neuroimaging procedures which are available for children with Down syndrome and which correlate with other protocol data are CT and magnetic resonance imaging (MRI) brain scans. Volumetric MRIs should be carried out periodically to assess deceleration of development, cerebellar growth, and the appearance of the superior temporal gyrus. When quantitative myelin studies are feasible through MRI, they may be of additional use.

Young adults (18–35 years of age) with Down syndrome have brain volumes which, when assessed quantitatively with CT, are smaller than those in age-matched controls. However, when brain volumes are normalized to body height (persons with Down syndrome are shorter than controls), there is no significant difference between subjects with Down syndrome

and controls (Schapiro et al. 1987). Thus, there is no in vivo imaging evidence of brain-growth reduction that is disproportionate to short stature in young adults with Down syndrome.

In older adults (more than 45 years of age) with Down syndrome who are demented, quantitative CT studies have demonstrated both increased cerebrospinal fluid (CSF) volume in cross-sectional studies and accelerated rates of dilatation of the lateral ventricles in longitudinal studies, as compared with age-matched controls and with older nondemented adults with Down syndrome (Schapiro et al. 1989b). The argument that neuroimaging analysis may correlate strongly with genotype is supported by the observation of Schapiro et al. (1989a) that an individual with mosaic Down syndrome, with three copies of chromosome 21q, was not mentally retarded but at age 45 years developed dementia, with an enlarged right lateral ventricular volume (as compared with mean ventricular volume in nondemented older individuals with Down syndrome) and with reduced metabolism in parietal association cortical areas (see below). Correlation of such findings with the neuropsychological battery of Haxby (1989) seems particularly promising. In the future, MRI will be preferred to CT for assessing volumes of intracranial structures in Down syndrome, as MRI provides better measures of gyral and lobar dimensions, being free of the CT bone-hardening artifact. CT, in which cross-sectional areas in the CT slices are summed by taking into account slice thickness and interslice separation, remains adequate for assessing lateral ventricular and whole-brain volumes (Schapiro et al. 1987, 1989b).

Data regarding positron emission tomography (PET) scanning in Down syndrome are new, and this procedure is not widely available at present. In young adults with Down syndrome who are not demented, there is a subtle abnormality in the correlations between "resting state" glucose metabolic rates in Broca's speech area and in frontal/parietal cortical regions (Horwitz et al. 1990), but absolute glucose metabolic rates do not differ from values in age-matched controls (Horwitz et al. 1990). In older demented adults with Down syndrome, glucose metabolic rates are reduced in parietal and temporal motor areas. These reductions are not found in young (18–35 years of age) or older (more than 35 years of age) nondemented adults with Down syndrome but are identical to PET-metabolic reductions in patients with Alzheimer disease in the population with Down syndrome (Schapiro et al. 1988).

Neuropathology

An understanding of the significance of the structural data concerning the development of the brain in Down syndrome ultimately depends on neuropathological observations. The following features of Down syndrome neuropathology are characteristic: (1) foreshortening of the frontal/occipital diameter of the forebrain (Zellweger 1977), (2) narrowing of the superior temporal gyrus (Kemper 1988), (3) disproportionately small cerebellum and brain stem (Crome et al. 1966), and (4) normal or nearly normal brain weight and head circumference at birth, with slowed postnatal growth rate in both of these parameters (Roche 1966; Benda 1971). Quantitative MRI can document the first three characteristics at any age, and the sequential pathology of characteristic 4 is best seen with serial MRI at birth–6 years. A characteristic feature of the cerebral cortex is a decrease in the small neurons in all cortical layers (Ross et al. 1984). This can be seen only at autopsy study and requires age-matched control material taken from the same areas as the samples taken from the Down syndrome brain, with processing in an identical manner.

An underlying assumption is that age-related atrophic changes in the brain occur prematurely in Down syndrome (Zellweger 1977; Kemper 1988), particularly in subjects who begin to demonstrate dementia (Schapiro et al. 1988, 1989b). These changes include mineralization of the globus pallidus, dilatation of the lateral and third ventricles, and the accumulation of senile plaques and neurofibrillary tangles in the cerebral cortex, hippocampus, and the amygdala. The dilatation of the ventricles and mineralization of the basal ganglia can be documented during life by MRI or CT scans in those who are more than 35 years old. The demonstration of senile plaques and neurofibrillary tangles depends on the use of special stains in selected brain regions at the time of autopsy and occurs in subjects older than 35 years. Their presence in large numbers is considered to be evident for Alzheimer disease in individuals with Down syndrome (Wisniewski et al. 1985).

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