Ataxia-Telangiectasia: Diagnosis and Treatment

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Much progress has been made in the early diagnosis of ataxia-telangiectasia since the gene was cloned in 1995. A clinical diagnosis can now be confirmed by radiosensitivity testing (colony survival assay), immunoblotting, and mutation detection. The diagnostic value of serum alpha-fetoprotein levels and radiosensitivity has been reevaluated using patients with diagnoses based on the presence of mutations in the ATM gene and the absence of ATM protein in nuclear extracts. Little progress has been made in treating the progressive ataxia. © 2003 Elsevier Inc. All rights reserved.

In memoriam: Robert A. Good, M.D., Ph.D. (1922-2003)

ATAxia-TELANGIECTASIA (A-T) is a progressive neurodegenerative disorder with onset of unsteadiness and truncal ataxia usually before 3 years of age.1-3 Head-tilting after age 6 months is also occasionally noted. By age 5, speech becomes slurred. Ocular apraxia is present in almost all patients with A-T, although it is difficult to elicit in very young children and is sometimes missed. Ocular apraxia in infants is occasionally misinterpreted as petit mal seizures. By age 10, the typical A-T child needs a wheelchair, usually because of excessive falling, coupled with slow reflexes, that causes serious bodily injuries. In the third decade of life, the neurologic deterioration seems to slow somewhat and/or the patient learns to adjust to the handicaps in various ways. One-third of patients have severe immunodeficiencies, accompanied by severe sinopulmonary infections with nonopportunist organisms; one-third are moderately impaired, and one-third have hardly any documentable immunodeficiency. Conjointival telangiectasias usually appear several years after the neurologic symptoms begin and also can be seen on the neck and ears in many children.

General handicaps include poor diction, handwriting, reading, and mobility, as well as a lifelong dependency for personal hygiene and bathing. Mental status is normal in most cases, with many patients having completed a university-level education. Life expectancy varies, with some patients living into the sixth decade. The three main causes of death are malignancy, infection, and nonspecific pulmonary failure. Although many malignancies are treated successfully, the most severe, such as acute lymphocytic leukemias, are often fatal at a young age. The pulmonary failure is probably secondary to repeated aspirations due to poor swallowing coordination. A few patients have had fatal reactions to radiation therapy for their malignancies.5 Because the malignancy sometimes precedes any obvious neurologic impairment, it is recommended that a diagnosis of A-T be considered in all young patients presenting with cancer, before radiation therapy is administered.

DIAGNOSIS

The clinical diagnosis becomes most apparent after age 10 years, when ataxia, apraxia, telangiectasia, and dysarthria are fully expressed. By this time, the disorder will be clearly progressive and may also be apparent in other siblings or relatives, indicating a genetic disorder of autosomal recessive pattern. By this age, cerebellar atrophy is also apparent on magnetic resonance imaging (MRI) studies.

In contrast, in very young infants the diagnosis can be elusive and easily confused with mild cerebral palsy, acute infectious or episodic ataxia, ataxia with oculomotor apraxia malignancy, or even other rare genetic or mitochondria disorders. Cerebellar atrophy is usually not apparent on MRI in young patients. A-T is the most common autosomal recessive ataxic disorder in children under age 5. Friedreich’s ataxia usually presents later in childhood, with noticeable absence of deep tendon reflexes and with positive Romberg’s and Babinski’s signs. It is in these very young patients that laboratory diagnosis is most helpful. Early diagnosis can play a major role in family planning, and also can help avoid years of costly diagnostic testing.
Three routine tests are immediately available to support a diagnosis of A-T in a young child: serum alpha-fetoprotein (AFP); karyotyping, with special attention to translocations involving chromosomes 7 and 14; and immune status of B-cell and T-cell compartments. AFP is elevated in >95% of A-T patients.6 False-positive findings are rare; however, in children under age 2 years, AFP may remain slightly elevated from the neonatal period. A very rare condition of hereditary persistence of elevated AFP can be ruled out by testing the parents. Elevated AFP is also associated with some malignancies. Karyotyping is seldom normal in A-T cells; however, mitogen-stimulated A-T lymphocytes may have to be harvested slightly later than usual, to visualize a sufficient number of metaphases for a careful analysis. T-cell levels are usually low, although many patients show only marginal deficiencies. Gamma/delta T-cell levels are usually elevated, probably reflecting a maturation defect in this pathway. B-cell levels are normal or slightly elevated. Serum immunoglobulin levels reveal marked deficiencies of IgE (in 80% of patients), IgG2 (in 80%), and IgA (in 60%). None of these parameters changes with disease progression. Serum IgM levels, in contrast, are highly variable, may change during disease progression, and sometimes rise to levels that necessitate treatment for hyperviscosity.3

Radiosensitivity is also characteristic of A-T cells7 and has been used to confirm the clinical diagnosis. However, only one radiosensitivity assay, the colony survival assay (CSA), has been validated for clinical diagnostic testing.8,9 The CSA is performed on Epstein-Barr virus–transformed lymphoblastoid cells (LCLs); this slows the laboratory turnaround time to 3 months. The CSA identifies radiosensitivity to 1 Gy in approximately 90% of patients with A-T (Fig 1); the remaining patients are also radiosensitive if tested with increased doses of ionizing radiation.9 LCLs from patients with various other disorders are also radiosensitive by CSA;10 of these disorders, only Mre11 deficiency manifests an ataxia of early onset that might be confused with A-T (see below). LCLs from five patients with Friedreich’s ataxia

Fig 1. CSA. Survival fractions <21% are considered radiosensitive; those above 36% are normal. Approximately 7% of bona fide A-T patients score in an intermediate range when tested only at 1 Gy; however, at 1.5 and 2 Gy they are also radiosensitive.9 Photo (lower right) shows the effects of conventional doses of radiation treatment to an A-T patient with lymphoma.
were not radiosensitive, nor were LCLs from four patients with early-onset ataxia with ocular apraxia (AOA1). Chromosomal breakage studies after in vitro irradiation also demonstrate this radiosensitivity; however, reliable diagnostic ranges for such testing have not been defined.

The defective protein in A-T is known as ATM. Intracellular ATM protein can be detected by immunoblotting (ie, Western blot) of extracts from normal cells. This test is difficult to perform on small amounts of blood; approximately 10 to 20 million cells are needed, because normal intracellular ATM protein concentrations are low. Thus immunoblotting is not practical for testing samples from young infants unless a cell line is first established. LCLs can then be tested for radiosensitivity as well. When nuclear extracts of LCLS are studied, the ATM protein is absent in >95% of patients with A-T (Fig 2). Very small amounts of ATM protein (<15% of control levels) are seen in the remaining few patients. As a control for whether equal amounts of protein are loaded into each lane of a gel, an antibody for Mre11 protein is used to develop the blots after the ATM protein level has been assessed; this also serves to identify possible patients with Mre11 deficiency. AOA1 can also be diagnosed by developing the same western blot with an antibody to aprataxin.

In rare cases (<1%), patients with A-T have been found to have normal amounts of ATM protein. In these patients the protein is functionally impaired, as can be demonstrated by analyzing one of the many targets (substrates) of ATM kinase activity to determine whether the expected serine or threonine residues have been phosphorylated. Either in vivo or in vitro assays of PI-3 kinase activity can be used. The most commonly tested ATM substrate is p53, which is phosphorylated by ATM on serine 15. Our studies of more than 40 A-T patients with or without detectable ATM protein have shown that typical A-T cells do not phosphorylate p53, nor do they phosphorylate alternate substrates such as MDM2 or nibrin. On the other hand, patients with some detectable ATM protein or with some detectable PI-3 kinase activity may have milder disease, with slower neurologic progression and longer survival.

**GENETIC ANALYSES**

Milder disease also appears to correlate with certain types of mutations in the ATM gene. Because of the large size of the ATM gene, and the fact that a large variety (>400) of mutations occurs in all 62 coding exons of the gene, with no hotspots, direct sequencing is not yet a cost-effective approach for diagnosing A-T. Also, many polymorphisms are encountered in any large gene sequence; these serve as false-positives in such testing, and each one needs to be identified and distinguished from a mutation.

A-T patients who are not from consanguineous matings carry two different mutations in the ATM gene, one mutation from each parent; that is, they are compound heterozygotes. Most mutations (>85%) result in failure to make ATM protein. Despite this, virtually all A-T patients have abundant amounts of ATM mRNA. Most mutations lead to premature termination codons, or mutations that delete portions of the message. Also included in this group of mutations are those that alter splicing motifs and result in the skipping of certain exons. A few rare mutations involve the insertion of small regions of intron into the mRNA (pseudoexons), again resulting in frame shifts and premature termination codons. Less than 10% of mutations in A-T patients are missense mutations; these change the amino acid sequence. However, the current understanding of the genetic code is imperfect, and some missense...
mutations may in fact be altering how the RNA is spliced; they may be altering exon splice enhancer and exon splice silencer motifs. Thus, the frequency of true missense mutations in A-T patients is probably even lower than that reported in the literature to date.

Because the ATM gene is so large and the mutation spectrum is so diverse, various mutation screening strategies have been used. Mutations are detected mainly by serial testing of the DNA and RNA from LCL cells. However, even before this is done, our laboratory first screens the DNA by determining the haplotypes across the ATM region. Four very informative microsatellite (short tandem repeat) markers are used: S1819, NS22, S2179, and S1818. The alleles for each marker have been carefully standardized so that haplotypes can be compared with a global database of affected haplotypes from >600 A-T patients (see also http://www.benaroyaresearch.org/bri_investigators/atm.htm). Some stratification of mutations is also possible by haplotyping for the very limited single nucleotide polymorphism haplotypes across the region of the ATM gene. If an affected haplotype is recognized, then the associated mutation is quickly checked by single-strand conformational polymorphism (SSCP) or direct DNA sequencing. Previously characterized haplotypes are identified in ~30% of new patients, although this figure approaches 90% for certain well-studied ethnic groups, including populations of Costa Rica, Norway, Spain, Brazil, Poland, and Turkey. This is also true for well-studied religious isolates, including Amish, Mennonites, and Moroccan Jews. This figure decreases as the heterogeneity of a population increases. Heterogeneity is high in American A-T patients.

If mutations are not identified by haplotyping, then RNA is collected from the patient’s LCL, converted to cDNA, and screened first by protein truncation testing (PTT). This method is also gel-based and can efficiently analyze fragments of ~1500 nt. In this way, by studying eight overlapping ~1500 nt fragments, the entire ATM gene cDNA (9168 nt) can be screened in a single experiment for regions that might contain truncating mutations, that is, those leading to premature stop codons. This method will not detect missense mutations and generally detects about 70% of all ATM mutations in A-T patients. (FitzGerald et al showed that PTT is not an efficient method for screening the mainly missense ATM mutations that occur in breast cancer patients.)

If mutations still remain undetected in the patient, then the cDNA is next screened by SSCP; this method is efficient for detecting all types of mutations in fragments smaller than 300 nt. SSCP involves screening 34 overlapping fragments. Twenty patients can be screened for three fragments on a single gel. The detection efficiency of SSCP approximates 80%.

Denatured high-performance liquid chromatography (dHPLC) uses genomic DNA and is a more automated screening method, involving capillary gel electrophoresis. The 62 coding exons are polymerase chain reaction (PCR)-amplified and are allowed to form heteroduplexes, which would arise if the patient were heterozygous for a mutation in a particular region of the gene. The fragments cover each of the coding exons and also include approximately 50 nt before (upstream) and after (downstream) of each exon, so as to also screen for mutations in the splicing regions of the DNA. If a patient is from a consanguineous mating, then normal DNA is mixed with patient’s DNA so that heteroduplexes can form at the mutated site. The efficiency of dHPLC for detecting ATM mutations approximates 85%. This method of screening for unknown ATM mutations needs further refinement before it can be reliably used for clinical application.

Whenever either of these methods detects an abnormal pattern, the suspect region ultimately must be sequenced across that region to identify the mutation. When all of the aforementioned methods are used in tandem, the detection efficiency approximates 95%. The missing 5% probably reflects that fact that all of these methods depend on PCR amplification across exons and the immediately adjacent sequences, but do not analyze large stretches of intronic sequences or the upstream or downstream regulatory regions of the gene. This is generally true for most large genes.

The task of trying to correlate specific mutations with clinical phenotypes is about to begin in earnest now that both mutations have been determined in a substantial number of A-T patients. To date, only a handful of mutations appear to correlate with phenotypic variations from the classical A-T syndrome. These include IVS40+1126A>G and the 7271T>G mutations. As mentioned earlier, both of these are associated with slower pro-
gression of neurologic symptoms, extended life span, and possibly reduced radiation sensitivity. Several other such associations have been suggested, but have not yet been well characterized.

**ATM FUNCTION**

The ATM protein has only one known function at the present writing; it is a serine-threonine kinase. It phosphorylates almost two dozen distinct substrates, including itself and p53, MDM2, CHK2, nibrin, H2AX, SMC1, Pin2/TRF1, FANCD2, BRCA1, IkBa kinase, and p53BP1. These substrates function in cell signaling to control the cell cycle, repair double-strand DNA breaks, respond to oxidative stress, and regulate transcription. ATM may also play a role in nonsense-mediated decay, perhaps by phosphorylating Upf1, because abnormal ATM cDNA is not degraded in A-T cells. It may phosphorylate the transcription factor AP-1. However, its roles in determining hypersensitivity to ionizing radiation or in neuronal development are not understood. The latter may involve protein degradation by ubiquination. Its role in the formation of telangiectases also remains unclear. (For a more detailed discussion of ATM-dependent functions, see Shiloh.)

**PATHOGENESIS**

The neuropathology of A-T is extensive, but at the same time selective, affecting neurons within the central nervous system as well as the spinal cord. The most pronounced degenerative changes are seen in the cerebellum, the area of the brain that coordinates both balance and timing. At autopsy, A-T patients have gross cerebellar atrophy due to a marked loss of Purkinje cell and granule neurons (Fig 3). Few Purkinje cells remain, and those that do typically exhibit an abnormal dendritic arborization with loss of secondary and tertiary dendrites. The presence of basket cell processes indicates that Purkinje cells are present initially, but deteriorate during the course of the disease. Ectopic cells are routinely found within both the molecular and granule cell layers, indicating migrational defects during the last trimester of gestation. It is presently unclear whether the loss of granule neurons is a direct result of the lack of ATM protein or is secondary to the loss of their synaptic targets (Purkinje cells). Purkinje cells of A-T patients are also characterized by shrinkage of the cell soma and disruption of axons, visualized as characteristic "torpedo" formations that are also seen in other ataxic disorders. The interneurons within the molecular layer and the neurons of the deep cerebellar nuclei are also reduced in number. Increased numbers of Golgi cells, glial fibrillary acidic protein–positive astrocytes, and CD68-positive microglial cells are detected. Reactive astrocytes and activated microglial cells are distributed throughout the cerebellum and suggest an immune response to the neurodegeneration.

Selective neuronal populations of the cerebellar afferent and efferent pathways are also affected, including those within the medulla, pons, and spinal cord. Specifically, loss of inferior olivary and pontine neurons, as well as demyelination and gliosis of the dorsal columns of the spinal cord are observed. Interestingly, many areas of the brain, including the neocortex, are spared.

Several questions remain in terms of understanding the pathogenesis that gives rise to the ataxic phenotype. One critical question is the role of the ATM protein in postmitotic neurons. It is known that the ATM protein functions to recognize DNA damage and signal the appropriate repair proteins that facilitate DNA repair. Consistent with this function, in situ hybridization has shown that ATM mRNA is confined to subpopulations of cells within highly proliferative zones of the brain early in mouse development (Fig 4). ATM expres-
sion is at its peak at embryonic time points when early cerebellar and other neuronal precursors are being generated. One can imagine the necessity for DNA damage surveillance in these proliferating neuroepithelial cells, which later migrate throughout the brain and become multiple types of neurons. At postnatal time points, ATM expression in the brain is quite low, even in Purkinje cells, where one might expect to detect increased levels due to the pathology seen in A-T patients. It has yet to be demonstrated whether ATM has the same function within postmitotic neurons, but some evidence has suggested that the ATM protein may play a neuroprotective role in tissues undergoing oxidative stress and apoptosis, probably secondary to its role in the activation and stabilization of p53.38,39

CANCER SUSCEPTIBILITY

Approximately one-third of A-T patients develop a malignancy during their lifetimes.40 Most of these malignancies are lymphomas or leukemias.40-42 Younger children tend to develop common acute lymphocytic leukemia, whereas older children sometimes develop TCL-1 expressing T-prolymphocytic leukemia (formerly called T-CLL).43 Both T-cell and B-cell lymphomas occur. Older patients also develop nonlymphoid cancers, such as stomach, breast, basal cell, ovarian, liver, uterine, and melanoma.44 Therapy is complicated by the fact that conventional doses of radiation therapy, as well as chemotherapy with radiomimetic agents, have been associated with adverse and sometimes fatal reactions (Fig 1).3,5

Much has been written about the cancer susceptibility of carriers or ATM heterozygotes, with suggestions that ATM heterozygosity may account for about 5% of familial breast cancers.45,46 This would be much greater than the 1% to 2% that is attributable to mutations in BRCA1 and BRCA2/FANCD1.47 ATM mutation frequencies vary from 2% to 29% in breast cancer cohort studies, with relative risks approximating four times that of the general population. However, estimates of risk are influenced greatly by study design, age of onset of cancer, and penetrance estimates—and large statistical confidence intervals are often overlooked in interpreting such results.

It is not yet clear which ATM mutations or types of mutations are associated with breast cancer susceptibility. The frequency of missense versus nonsense mutations in breast cancer cohort studies (15% nonsense versus 85% missense) appears to be essentially reversed from the types of mutations seen in A-T patients (>90% nonsense versus <10% missense). Furthermore, the ATM heterozygotes identified in such breast cancer cohort studies do not seem to have relatives with A-T. Thus the study design seems to influence the types of ATM mutations found. A model was suggested that heterozygosity for at least some missense mutations may result in a dominant negative effect whereby an abnormal ATM protein can be more detrimental than having no protein at all, as is seen with null or nonsense mutations in most A-T patients.46

When missense mutations were created in “knocked in” mouse models, an increased frequency of tumors was seen in heterozygous mice (as well as in the homoyzogotes, of course).48,49 This finding was in stark contrast to the absence of tumors in mice that were heterozygous for “knockout” mutations and expressed no ATM protein.50 Thus dominant negative missense ATM mutations may play a much greater role in cancer susceptibility in the general population than was first appreciated. This is also supported by the mutagenesis experiments of Scott et al.51 When all of the foregoing facts are considered together, the frequency of ATM carriers in the general population could exceed 5%, as compared with the 1% carrier frequency estimates that were based on patients and families affected with A-T.52 On the other
hand, carriers of missense mutations do not appear to manifest any obvious neurologic symptoms.

RELATED DISORDERS

Several A-T–like disorders have been described. However, of these, only Mre11 deficiency (also called A-T–like disorder) has ataxia with cerebellar degeneration and is radiosensitive in diagnostic testing, and thus might be misdiagnosed as A-T.13,53 Because Mre11 deficiency is very rare (three families have been reported, and few of the clinical details have been forthcoming to date), it is difficult to generalize about the clinical picture. However, in at least one family, both affected siblings were still ambulatory in their twenties; this would not be typical of A-T. Because Mre11 appears to influence the upstream activation of ATM itself and also forms a DNA repair complex with Rad50 and nibrin, which is phosphorylated by ATM in response to radiation damage, it is not surprising that the phenotypes of Mre11 deficiency and A-T overlap. In contrast, however, nibrin is deficient in Nijmegan Breakage Syndrome (NBS) (formerly called A-T variant 1) and NBS patients do not have ataxia; they are usually microcephalic and mentally impaired—findings uncommon in A-T patients.54 Telangiectases have not been observed in either Mre11 or NBS patients. NBS patients manifest both in vitro and in vivo radiosensitivity.9,55 All three disorders manifest an increased frequency of translocations involving chromosomes 7 and 14 in peripheral blood lymphocytes. The G1/S checkpoint is also impaired in all. ATM proteins levels are normal in Mre11 deficiency and NBS patients.

Recent genetic studies of patients with early-onset ataxias and oculomotor apraxia suggest that at least two distinct forms exist: 1) AOA1 results from mutations in the aprataxin gene located on chromosome 9p1369-73 and includes patients with Aicardi syndrome.74 2) The second form links to a gene on chromosome 9q34.75 Both CSA and western blotting can be used to distinguish AOA1 from A-T.

TREATMENT

There is nothing more discouraging for a patient or family member than to be given a specific diagnosis of A-T and then be told that there is no effective therapy to halt the progressive ataxia. Over the past 5 years, molecular genetic research has completely revolutionized how the pathophysiology of A-T is understood, but this has yet to produce any effective neuroprotective or neurorestorative therapies.

The treatment of A-T remains based in medical management (of immunodeficiencies and sinopulmonary infections, neurologic dysfunction, and malignancy) and neurorehabilitation (physical, occupational, and speech/swallowing therapy; adaptive equipment; and nutritional counseling). Modest additional gains have been made in the use of medications that can improve imbalance, incoordination, or dysarthria (such as amantadine,56 buspiron,57 and fluoxetine58); cerebellar tremor (with clonazepam, gabapentin,59 and other anticonvulsants, such as propranolol); and basal ganglia dysfunction (with L-dopa compounds, direct dopamine agonists, and anti-cholinergics). The latter may also help with drooling. However, side effects of some medications (eg, drugs used in cancer treatment) may in turn worsen the ataxia. Prednisone is often used to treat malignancies in A-T patients, and has been associated with decreased severity of ataxia; however, this effect is temporary, and the long-term side effects of steroid treatment for ataxia outweigh the modest potential benefits.

As in essential tremor, botulinum toxin injections to selected muscles have been used to control cerebellar-mediated tremor of the head or limbs. Muscle weakness (including dysphagia from neck injections) can be a bothersome complication that can last up to 3 months. Surgical therapies for tremor include thalamotomy and thalamic stimulation, the latter being better tolerated, but not without risk. Costly interventional procedures should be reserved for the most disabling cases that are unresponsive to medical management.

Secondary complications of progressive ataxia can include deconditioning/immobility, weight loss or gain, skin breakdown, recurrent pulmonary and urinary tract infections, aspiration, occult respiratory failure, and obstructive sleep apnea, all of which can be life-threatening. As maturing patients come to realize their physical and social limitations more fully, they often develop depression and hostile feelings toward caregivers. These emotional problems should be recognized and addressed.

Symptomatic treatment can greatly improve the quality of life of these patients and prevent com-
lications that could hasten death. Supportive interventions—including education about the disease, genetic counseling, individual and family counseling, referral to support groups and advocacy groups, and guidance to online resources—should always be offered. Misinformation, fear, depression, hopelessness, isolation, and financial and interpersonal stress can often cause more harm to the patient and his caregiver than the ataxia itself.

Treatment of the symptoms of cerebellar ataxia should be symptom-focused (imbalance/incoordination/dysarthria, cerebellar tremor) and monitored with a few simple reproducible and semiquantitative measures of performance (ie, timing of gait, single-limb balance, hand and foot tapping, completion of a spiral maze or pegboard, reading of a standard paragraph). Self-reporting of functional measures (eg, use of aids for gait, number of falls, success at a keyboard or electronic game or carrying a cup of coffee across a room, need for assistance with dressing or personal care or eating, difficulty being understood) can also be reliable indicators. Crawford et al. have been developing standard paragraph (im

REMARKS

37. Vinters HV, Gatti RA, Rakic P: Sequence of cellular events in cerebellar ontogeny relevant to expression of neuronal abnormalities in ataxia-telangiectasia, in Gatti RA, Swift M (eds): Ataxia-Telangiectasia: Genetics, Neuropathology, and Immunology of a Degenerative Disease of Childhood. New York, Alan R Liss, 1985, pp 233-255