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Ataxia-Telangiectasia: Closer to Unraveling the Mystery

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Abstract

Ataxia-telangiectasia (A-T) is a progressive genetic disorder affecting the central nervous and immune systems, and involving chromosomal instability, cancer predisposition, radiation sensitivity and cell cycle abnormalities. Studies of the cellular phenotype of A-T have pointed to a defect in a putative system that processes a specific type of DNA damage and initiates a signal transduction pathway controlling replication and repair. A-T is genetically heterogeneous, with 4 complementation groups. While functional cloning of the A-T gene(s) using gene transfer has proven problematic, positional cloning attempts are zeroing in on a defined interval on chromosome 11q22–23 that probably harbors the mutations for all 4 complementation groups.

Ataxia-Telangiectasia: A Pleiotropic Defect in an Essential Junction of Cellular Physiology

Dr. Elena Boder's review [1] on the genetic disorder ataxia-telangiectasia (A-T) 10 years ago began with the words: 'Ataxia-telangiectasia has been mysterious from the start.' Since its establishment as a clinical entity in 1957 [2], this enigmatic, insidious disorder has presented a biological, medical and human challenge to clinicians and researchers. The mutations responsible for A-T seem to affect an unidentified physiological junction linking the differentiation of various tissues, essential functions in the central nervous and immune systems, genome stability, DNA replication, recombination and repair, cell cycle control, cellular aging and neoplastic transformation [3–8]. Hence, identifying the sites of these mutations is expected to have far-ranging effects in several areas of biomedical research.

A-T is inherited in an autosomal recessive manner and has been found worldwide, with

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patient frequencies of about 1:100,000 in the United States and Britain [9–12]. There are notable concentrations of A-T patients also in Turkey [13], Italy [14] and among Moroccan Jews in Israel [15].

A-T makes its appearance initially as a neurological disorder [1, 4, 5]. Cerebellar ataxia begins in infancy and progresses steadily, confining the patient to a wheelchair by the beginning of the second decade of life. Other main neurological signs are involuntary movements, diminished or absent deep reflexes, apraxia of eye movements and slurred speech. The neuropathological hallmark of A-T is cerebellar degeneration involving primarily the Purkinje and granular cells; degenerative changes have also been noted in the spinal cord and ganglia, brainstem and peripheral nerves. The second clinical hallmark of A-T, which typically appears between the ages of 3 and 6 years, is telangiectases (dilation of blood vessels making them more prominent) in the eyeballs and conjunctiva, sometimes spreading over sun-exposed areas of the skin. Some 50–80% of patients show the third clinical hallmark of A-T, recurrent sinopulmonary infections signifying marked immunodeficiency. Serum levels of IgA, IgG2 and/or IgE are reduced, the number of circulating lymphocytes is diminished and mitogen response is poor. The thymus is degenerated and sometimes absent. The serum levels of two oncofetal proteins – α-fetoprotein and carcinoembryonic antigen – are consistently higher in A-T patients. Somatic growth and sexual maturation are usually retarded, with female hypogonadism being almost uniform. Progeric changes typically appear in the hair and skin, marking premature senescence. Intelligence is usually normal.

Another cardinal feature of A-T is profound cancer predisposition, which becomes evident in about 10% of patients during childhood [16–18]. Lymphomas and acute lymphocytic leukemia constitute over 85% of all cancers in A-T and appear primarily in younger patients. Other cancers, mainly epithelial, rise steadily with age [10]. Early attempts to treat these malignancies by radiotherapy resulted in acute radiation reactions, revealing another feature of A-T – a profound sensitivity to the cytotoxic effect of ionizing radiation [19–24]. The course of A-T is progressive and relentless, and patients usually die with respiratory failure or malignancy during the second or beginning of the third decade of life. There is no effective way to retard the progression of this disease.

The primary diagnostic laboratory finding in A-T is chromosomal instability, evident as high rates of chromosomal breaks, usually observed in peripheral lymphocytes or fibroblasts [25–27]. Lymphocyte cultures show cell clones containing specific chromosomal translocations involving particularly the chromosomal regions 7p14, 7q35, 14q12 and 14q32 which harbor the T-cell receptor and immunoglobulin heavy-chain genes. Such clones often precede the onset of lymphoreticular malignancies and subsequently undergo clonal expansion as malignancy progresses [27–36]. Molecular analysis of several translocation breakpoints showed that the immune system genes residing in these regions were indeed involved in these aberrations [30, 32, 33, 35, 36].

The cellular phenotype of A-T further reflects the complexity of this disorder. Besides chromosomal instability, A-T cells show a reduced life span in culture, higher requirements for unspecified serum growth factors, abnormalities in the shape and arrangement of cytoskeletal actin fibers and abnormal content of a variety of extracellular surface proteins [37, 38]. A major cellular characteristic of A-T, which has become diagnostic, is the profound sensitivity of the cells to the cytotoxic and clastogenic effects of ionizing radia-

tion and radiomimetic chemicals. A-T cells are hypersensitive to both high- and low-energy transfer ionizing irradiation, as well as to a host of chemicals that mimic the effect of ionizing radiation on DNA by their capacity to produce radicals capable of inducing strand scissions [3, 39–51]. However, the overall kinetics of single- and double-strand break repair in A-T cells has been found normal in most studies [3, 39, 44, 49, 52, 53]. Unexpectedly, semiconservative DNA synthesis in A-T cells was found to be more resistant to the inhibitory effect of the DNA damaging agents to which A-T cells are sensitive, most notably ionizing radiation [54–56]. This phenomenon, called 'radioresistant DNA synthesis' (RDS), was the first evidence of a defect in cell cycle control in A-T cells (see below).

The clinical and cellular characteristics of A-T have been used to delineate phenotypic variants among patients. Patients with somewhat milder clinical signs, later age of onset and slower progression of the disease were found in several countries, and in some of them this phenotype was correlated with milder radiosensitivity and sometimes reduced or absent RDS. These parameters do not always coexist, however, demonstrating the complexity of the molecular and physiological basis of A-T [6, 8, 13, 57–65].

Another dimension is added to this complexity by other disorders with certain characteristics shared with A-T, such as immunodeficiency coupled with chromosomal instability [66–68]. The combination of microcephaly, growth retardation, immunodeficiency, chromosomal instability, radiosensitivity and RDS but no telangiectases has been particularly related to A-T. Patients with this syndrome, sometimes associated with mental retardation, were reported in several ethnic groups, and some were classified as Nijmegen breakage syndrome [69–77]. Patients with Nijmegen breakage syndrome tend to develop lymphoreticular malignancies at a higher rate than classical A-T patients [77]. One case of A-T with microcephaly and mental retardation was designated 'AT_{Fresno}' [78].

Attempts to delineate the possible genetic heterogeneity of A-T and its relationship with related syndromes have been done by fusing cells from different patients and measuring RDS [79–81] or radiation-induced chromosomal aberrations [82, 83] in heterokaryons. These studies revealed 4 complementation groups in classical A-T, designated A, C, D and E, and 2 complementation groups, V1 and V2, among patients with Nijmegen breakage syndrome and related syndromes [77, 81]. Groups A and C accounted for 83% of cases in a sample of 50 A-T patients [81], but the size of this series precludes making generalizations to A-T worldwide. In general, no correlation was found between complementation group assignment and clinical variation in A-T. It is unclear at present whether these complementation groups represent different genes or different mutations within 1 gene. A-T researchers cautiously refer to 4 genes involved in classical A-T: ATA, ATC, ATD and ATE.

A-T heterozygotes have always received special attention. In a sense A-T is not entirely recessive since carriers mildly manifest two of the disease characteristics, cancer predisposition and radiosensitivity. Epidemiological studies consistently show that A-T heterozygotes exhibit a higher rate of certain cancers, especially breast cancer in women [9, 11, 16, 84–88]. Swift et al. [86] estimated the cancer tendency among male A-T heterozygotes to be 3.8-fold higher than that of the general population, while that of female carriers was estimated to be 3.5 higher. However, the relative risk for breast cancer in women alone was estimated to be 5.1 higher than that of a control population. Swift et al. [84] further suggested that up to 8.8% of the American white female

patients with breast cancer may be A-T carriers. Easton [88] found in British and Norwegian populations a 3.9-fold risk for breast cancer in female A-T heterozygotes, while no tendency to develop other cancers was found among A-T carriers in general. These findings were accompanied by repeated reports of moderate sensitivity of cells from A-T carriers to ionizing radiation, as measured by survival and cytogenetic assays [89–108]. These results imply that A-T heterozygotes might face special hazards from routine diagnostic or therapeutic procedures involving radiation. These findings also stimulated attempts to develop laboratory assays for the detection of A-T carriers in the general population. While obligatory A-T heterozygotes could be clearly delineated from controls in some studies [89, 91, 94–98], groups of controls and A-T heterozygotes overlapped in other samples [92, 93, 101, 107]. The wide range of radiation responses among control groups makes the reliability of this parameter as an assay for carrier detection doubtful. It should also be noted that most of these methods are labor intensive and results vary between laboratories.

A-T: A Defect in Processing of a Specific DNA Lesion Affecting a Downstream Signal Transduction System

The function presumed defective in A-T is associated with the processing of a specific type of a DNA lesion. Examination of the mode of action of chemical agents to which A-T cells are hypersensitive pointed to a specific type of strand scission induced by these agents via a free radical attack on the deoxyribose moiety [40]. This high specificity should be borne in mind when attempting to make deductions about the A-T defect from the cellular phenotype. The high sensitivity of A-T cells to agents inducing this lesion has been attributed to a defect in DNA repair or in a mechanism controlling replication of damaged DNA [3, 5, 6, 39, 49]. It is noteworthy in this respect that the RDS phenomenon could be clearly dissociated from radiosensitivity in several reports [46, 59, 109–112]. Early observations that A-T cells are deficient in 'potentially lethal damage repair' acting in quiescent cells [113–115] seemed to support the DNA repair hypothesis. The natural candidate for the critical DNA lesion unrepaired in A-T cells was DNA breaks. Most studies based on biophysical methods have failed to reveal a defect in the overall kinetics of DNA strand break repair in A-T cells [3, 39, 44, 49, 52, 53, 116–118]. However, several investigators did notice an elevation in the residual amount of unrepaired strand breaks in irradiated A-T cells and suggested that these cells might handle the DNA strand break differently from other cells [119–122]. Cytogenetic studies also pointed to a residual amount of double-strand breaks unrepaired in A-T [112], or a higher fraction of double-strand breaks converted to chromosomal breaks [123–125]. A defect in chromatin structure was suggested to be responsible for this phenomenon [126]. Several studies pointed specifically to possible misrejoining of DNA breaks in damaged molecules introduced into A-T cells [120, 127–132] or treated with A-T cellular extracts [51, 133–135]. A recombination-based mechanism responsible for double-strand break repair and defective in A-T was suggested [51, 132] and related to in vivo observations of an increased rate of uncommon transrearrangements between T-cell receptor genes in A-T lymphocytes [136–138]. As expected, general V(D)J rejoining in A-T patients was found normal [139, 140]. Abnormal recombination in A-T was also noticed by Meyn [141], who observed an extremely high rate of intrachromosomal recombination in A-T cells, while interchromosomal recombination remained normal.

While the mechanism that directly handles the critical DNA lesion in A-T is still unclear, additional effects of the abnormality in this mechanism on downstream systems induced by DNA damage have been observed. The main observation consistently made in A-T cells is that several cell-cycle checkpoints activated by radiation damage do not function optimally. The postirradiation inhibition of the cell cycle that normally occurs at the G_1 and S phases was found to be less pronounced in A-T cells, while the G_2 phase in A-T cells irradiated at G_1 or S was more prolonged than in normal cells. But, when A-T cells were irradiated at G_2, the delay in traversing this stage into mitosis was shorter than in normal cells [102, 142–153]. Radiation-induced G_1 arrest is mediated by a newly revealed signal transduction system involving a rise in the cellular level of the p53 protein, probably via a post-translational control mechanism [153–156]. p53, in turn, induces the expression of several genes including Gadd45, $p21^{WAF1/CIP1}$ and Mdm2 [155, 157–163]. The products of Gadd45 and $p21^{WAF1/CIP1}$ inhibit DNA replication, while the Gadd45 protein also stimulates DNA repair [157, 159, 160, 164]. This system is perturbed in A-T cells of all complementation groups when stimulated by ionizing radiation or radiomimetic chemicals but functions normally after treatment with UV irradiation and other agents to which A-T cells are not sensitive [151, 152, 155, 164–166]. It has been proposed, therefore, that several signal transduction pathways activating p53 may be induced by specific types of DNA damage, with the one activated by strand breaks being defective in A-T cells [165, 167].

The p53 gene product mediates another radiation-induced pathway leading to programmed cell death (apoptosis) [168–170]. Meyn et al. [171] noticed that A-T cells sustain higher rates of apoptosis following irradiation, while disruption of p53 function increases their radioresistance. It was proposed that A-T cells have an unusually low threshold for triggering p53-mediated apoptosis and that their radiosensitivity stems from induction of apoptosis by usually nonlethal doses.

The DNA repair hypothesis and the DNA replication/cell cycle hypothesis could be merged by assuming that A-T cells may harbor a defect in a protein or a protein complex involved in both the initial processing of a specific DNA strand break and in triggering the signal transduction system leading to G_1 arrest and enhancement of DNA repair. A defect in this system might result in unrepaired breaks leading to chromosome damage and possibly to the dominance of an error-prone repair system of lower fidelity which is usually overshadowed by the regular repair mechanism. The function of the downstream systems activated by that protein is reduced, with consequent cell cycle abnormalities. Another obvious possibility that cannot be ruled out at this point is a defect in a transcription factor responsible for the normal action of several genes controlling these systems.

Complementation Cloning Attempts: Too Many Genes Volunteering for the Same Job

The common approach to the identification of disease genes with yet unrecognized protein products is positional cloning [172, 173]. The localization of the A-T locus to chromosome 11q22–23 [174] opened the way to application of this approach to A-T (see below). But the cellular phenotype of A-T lends itself to another strategy for gene identification – functional cloning by complementation of the cellular phenotype.

Hypersensitivity to DNA-damaging agents, a prominent feature of A-T cells, calls for attempts to complement this phenotype by gene transfer. In this approach, exogenous DNA is introduced into the cells and selection applied to identify cell clones in which this sensitivity has been 'corrected'. An attempt is then made to identify the piece of DNA supposedly responsible for this effect, which is expected to represent a normal allele of the disease gene. Functional cloning by this strategy is appealing since it circumvents the more labor-intensive positional cloning. Functional cloning proved particularly useful when rodent cell mutants sensitive to specific radiations or chemicals were transfected with human genomic DNA or cDNA; these experiments led to isolation of a host of human genes involved in transcription and DNA repair, some of which are involved in specific complementation groups of the UV-sensitive disorders xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy [175]. Application of this strategy to human cells is experimentally more difficult but has been successful in isolating the genes for complementation groups A and C of xeroderma pigmentosum [176, 177] and Fanconi's anemia group C [178].

Several laboratories have invested considerable effort during the last decade in applying this approach to A-T. Early trials to complement the radiosensitivity of A-T cells were based on transfection with genomic DNA. In this system, pieces of the exogenous DNA are expected to integrate into the cellular genome and be stably expressed. Lehmann et al. [179] and Green et al. [180] transfected the immortalized A-T(D) cell line AT5BIVA with human or mouse genomic DNA together with the selective marker gpt. One radioresistant cell clone was obtained out of 400,000 gpt+ transfectants and showed a normal level of radiation resistance but only partial correction of RDS. Attempts to rescue the responsible DNA fragment were unsuccessful. Lohrer et al. [181], using the same cell line in similar experiments, obtained no stably radioresistant transfectants. It was concluded that limiting factors, in particular the size of genomic DNA stably integrated into the genome of A-T cells, may prevent the success of experiments based on the use of such A.

Despite this discouraging conclusion, Kapp and Painter [182] transfected AT5BIVA cells with a genomic library in a cosmid vector and were able to identify a transfectant clone in which cellular and chromosomal radiosensitivities were corrected to an intermediate level, but RDS was retained. Subsequent isolation of the integrated DNA [183] revealed genomic sequences that mapped to the chromosomal band 11q23, to which the A-T locus had previously been linked [174]. A 3.0-kb cDNA clone corresponding to these DNA fragments was isolated and found to map near the THY-1 gene, now known to be located some 25 cM distal to the A-T locus (see below). This gene, termed ATDC (AT-D complementing), codes for 9 alternatively spliced transcripts with variable patterns of expression in different tissues [183, 184; Kapp, pers. commun.] and is not induced by ionizing radiation [184]. The ATDC protein contains several zinc finger motifs and a leucine zipper domain, indicating possible formation of a homo- or heterodimer involved in nucleic acid binding, typical of regulatory proteins [185]. Murnane et al. [186] used the two-hybrid system in yeast to demonstrate that this protein indeed forms homodimers. No mutations in this gene have been identified to date in A-T(D) cells [Kapp, pers. commun.].

In order to avoid the use of genomic DNA, Ziv et al. [187] chose to introduce a cDNA library cloned in the expression vector pCD [188] into the A-T(A) cell line AT22IJE-T

[189], expecting the small size of the cDNA inserts to contribute to their stable integration and maintenance in the cellular genome. Out of 200,000 transfectants, 2 cell clones showed partial correction of radiomimetic sensitivity and RDS. Attempts to rescue the integrated cDNAs failed, however, probably due to dissociation between the inserts and the vector sequences used to identify the integrated DNA pieces.

While these results were not encouraging, the notion that phenotypic complementation can be obtained in A-T cells by gene transfer did gain support from experiments with another technique – the microcell-mediated chromosome transfer. In this system, whole chromosomes tagged with a selective marker are introduced into the cells, and the introduced genes residing in their 'natural' environment are expected to remain intact. The assignment of the A-T locus to chromosome 11q22–23 by Gatti et al. [174] spurred experiments with this approach. Ejima et al. [190] and Komatsu et al. [191] showed that introduction of chromosome 11 indeed restored cellular radioresistance in different A-T cell lines and that the responsible gene was distal to 11q14 [192]. Chromosomal radiosensitivity was complemented in other experiments [193], and aberrant derivatives of chromosome 11 enabled localization of the responsible gene to 11q23 [194]. Lambert et al. [195] used a chromosome 18 derivative containing translocated material from 11q22–23 to show that this chromosomal region indeed contained a gene that complements three phenotypic features of A-T(D) cells: radiomimetic sensitivity, RDS and the abnormal postirradiation cell cycle kinetics.

Functional cloning by transfer of human DNA into human cells has always suffered from two drawbacks: the low uptake of exogenous DNA by human cells and the difficulty of direct identification of the introduced DNA against the background of the recipient's DNA. Both problems are largely eliminated when human DNA is introduced into rodent mutant cells that simulate the appropriate human mutations. In fact, most of the human genes involved in UV response identified using gene transfer were rescued from UV-sensitive rodent cells transfected with normal human DNA [175]. An extensive collection of X-ray-sensitive hamster mutants has been obtained in several laboratories [196]. Thacker and Ganesh [197] identified the mutant irs-2 obtained from V70 hamster cells, showing the same sensitivity profile and RDS like A-T cells, with no apparent defect in strand breakage repair [198]. Zdzienicka et al. [199] identified 3 V79 mutants with spontaneous chromosomal breakage, cellular and chromosomal radiosensitivity, RDS and normal strand break repair. Their sensitivity profile with regard to other DNA-damaging agents was remarkably similar to that of A-T cells [200]. Interestingly, all 3 mutants belong to the same complementation group as the irs-2 mutant [201]. Fusion of these cells with human HeLa cells resulted in full complementation of RDS [202]. However, introduction of human chromosome 11 containing an intact 11q22–23 region did not result in correction of the mutant phenotype, while the same chromosome complemented the phenotype of the A-T cell line AT5BIVA cells [203]. It was concluded that this phenotype, which so remarkably resembled the human A-T defect, was caused in the hamster cells by a gene unrelated to the human A-T gene [203]. Transfection of these mutants with HeLa genomic DNA or a human genomic cosmid library yielded transfectants that gained some radioresistance but retained RDS [204]. Microcell-mediated chromosome transfer of additional human chromosomes into these mutants showed that intermediate X-ray sensitivity could be conferred by human chromo-

somes 4 and 15, together with a mouse chromosome. The combination containing human chromosome 4 also fully complemented RDS [204]. These studies underscored again the dissociation between radiosensitivity and radioresistant DNA synthesis, and the genetic complexity underlying these two biological end points.

Chromosome-mediated gene transfer is advantageous as it facilitates separation between the recipient's genome and the introduced genes. It has the drawback, however, of focussing on a chromosomal region rather than on a discrete gene. A system based on episomal expression vectors that replicate in the cells as extrachromosomal elements allows the introduction and expression of individual cDNAs without their chromosomal integration. These vectors are easy to rescue and are attractive for use with A-T cells because they are presumed less vulnerable to the inherent genomic instability of these cells. Commonly used episomal vectors contain the origin of replication and EBNA-1 antigen gene of the Epstein-Barr virus. The binding of EBNA-1 protein to the origin of replication sequence enables episomal replication of the plasmid [205–208].

Three laboratories have recently used episomal cloning systems to identify cDNAs complementing the sensitivity phenotype of A-T cells of group D [209], group A [210] and group E [211], with remarkably similar results. In all cases, stable transfectants which had acquired various degrees of radiomimetic resistance were obtained, and episomal cDNAs were rescued and identified. A total of 26 cDNA clones were obtained in these 3 studies and found to confer different degrees of resistance to ionizing radiation or radiomimetic drugs upon repeated transfection. However, in the study of Ziv et al. [210], only 1 of 13 cDNA fragments that complemented the radiomimetic sensitivity of A-T(A) cells also partly corrected RDS. These results and previous observations point to the possibility of separating between these two features of the A-T phenotype [59, 109, 110, 182, 212].

An intriguing finding of these studies was that many of the complementing cDNAs were not full length and some represented only the 3′ untranslated regions of the corresponding cDNAs [211]. Chen et al. [211] suggested that 3′ untranslated regions of certain cDNAs may be able to modify the cellular response to radiomimetic agents by unknown regulatory mechanisms. This assumption implies that the phenotypic complementation system as a tool for studying the molecular basis of certain diseases may be highly prone to background noise, at least in the case of A-T. Indeed, all the cDNAs identified in these studies represented a large variety of genes: many were previously known, and none mapped to the A-T locus at 11q22–23. Several of the previously known genes, like phospholipase A2 (obtained independently by Ziv et al. [210] and Chen et al. [211]), and heat shock cognate protein 70 [210] are involved in various stress responses. The role of others, such as ferritin H chain, cytochrome C and ribosomal proteins [210], in cellular responses to radiomimetic sensitivity is unclear.

These studies led to the conclusion that the biological end points that define the two A-T phenotypic hallmarks – radiosensitivity and RDS – can be modulated by high expression of a number of sequences, not necessarily full-length transcripts. In such a situation complementation cloning may suffer from a low signal-to-noise ratio, since some of these cDNAs may even mask the effect of a clone derived from the disease gene itself. In view of these results, attempts to identify the elusive A-T genes shifted recently to positional cloning. Phenotypic complementation may still be helpful in testing the authenticity of a candidate gene obtained by that approach.

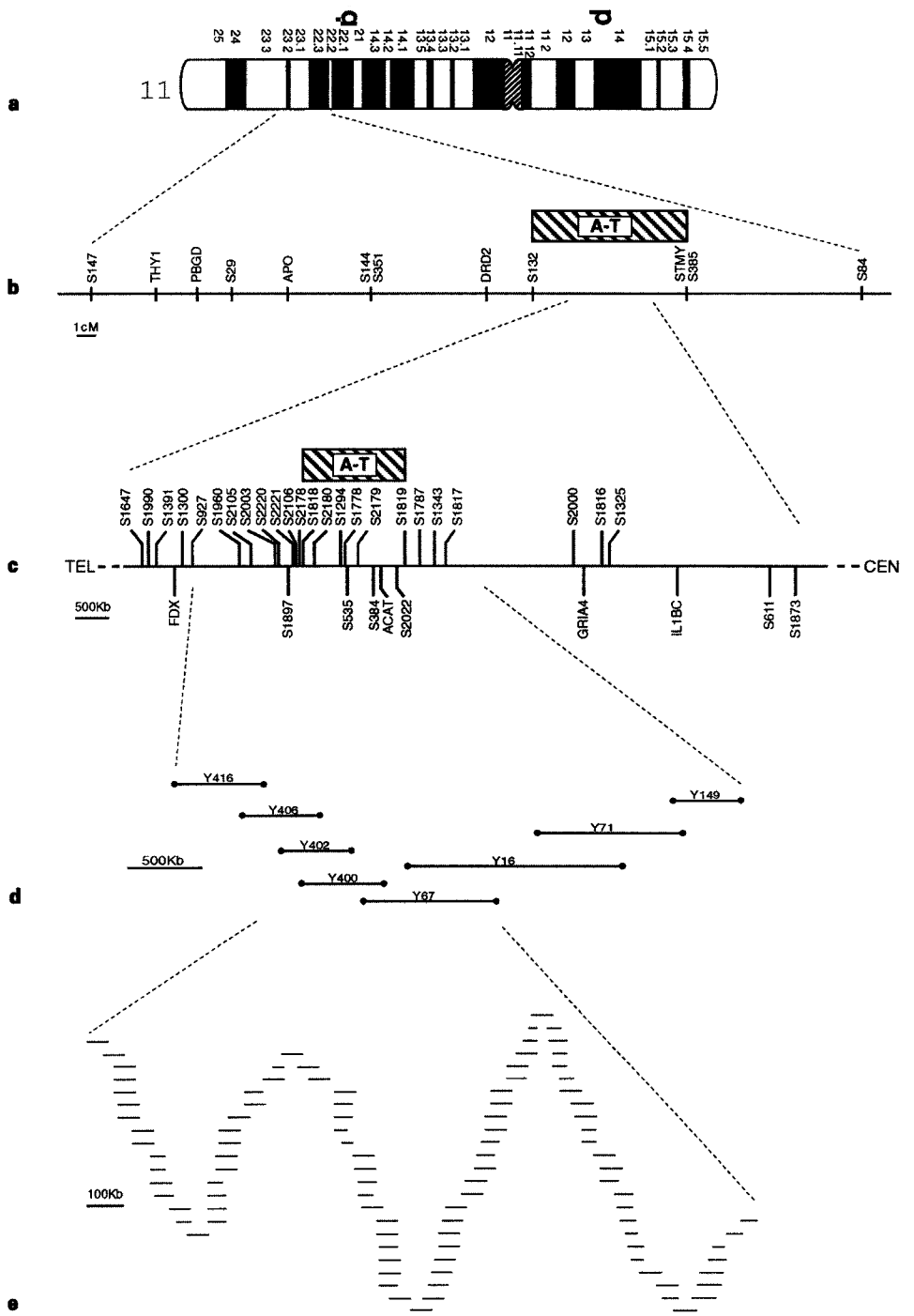
Positional Cloning: Zeroing in on the Culprit Genes

The well-established positional cloning paradigm [172, 173] has recently had impressive success with scores of disease genes, including some high-profile ones [173, 213–218]. The basic steps in this strategy include: the localization of a disease locus to a specific chromosomal region by linkage analysis; extensive generation of highly polymorphic markers in the region and narrowing the locus by genetic analysis; long-range cloning and physical mapping of the disease locus; identification of transcribed sequences ('gene hunting'), and, finally, a search of the candidate genes for mutations in patients. Attempts at positional cloning of the A-T genes have recently culminated in the construction of extensive transcript maps of the A-T locus and a search for mutations in a fair number of candidate genes.

The genetic heterogeneity of A-T presents a potential obstacle to linkage analysis should several A-T genes reside in different locations. This has proved to be the case in xeroderma pigmentosum and Fanconi's anemia [175, 219]. Gatti et al. [174] conveniently skirted this problem by conducting initial linkage analysis on a 61-member Amish A-T kinship assigned to complementation group A. The first marker that gave a lod score suggestive of linkage with A-T was THY-1 localized on chromosome 11, region 11q22–23 (fig. 1a). Further analysis with additional markers at this region and additional group A families substantiated this finding, and other unassigned A-T families appeared to make the 11q22–23 localization conclusive. This milestone in A-T research opened the way to positional cloning efforts. Additional studies on American, Turkish, British, Israeli and French families [220–225] clearly showed that a major A-T locus containing the group A gene resided at 11q22–23 but proximal to THY-1. These studies utilized increasing numbers of restriction fragment length polymorphism markers and refined genetic and physical maps of this region [222, 226–230] and did not indicate locus heterogeneity, thus pointing to a possible single A-T locus at 11q22–23. This assumption gained significant support from a linkage study conducted by Ziv et al. [231] with a single Moroccan Jewish A-T family assigned to group C. Significant lod scores obtained with 11q22–23 markers indicated the close proximity of the A-T(A) and A-T(C) mutations. A consortium-based analysis of 111 families from the United States, Turkey, England, Italy and Israel narrowed the disease locus to an 8-cM sex-averaged interval between the markers STMY and D11S132/NCAM [232] (fig. 1b). McConville et al. [233] later suggested that the group E locus may also be located in this interval, which probably spans the 11q22.3–23.1 boundary.

In the absence of a chromosomal aberration flagging the physical location of the disease gene, repeated genetic analysis was required to narrow the A-T search interval. McConville et al. [233] and Ambrose et al.

Fig. 1. Positional cloning of the A-T genes. Assignment of the A-T locus to chromosome 11q22–23 [174] (**a**) has led to a primary linkage map of the A-T region and confinement of the A-T locus within an 8-cM interval [232] (**b**). Subsequent construction of a high-density microsatellite map of the region [242] and repeated-linkage analysis [245, 246] determined an interval between D11S1818 and D11S1819 probably containing the mutations for all 4 complementation groups of A-T (**c**). According to Vanagaite et al. [242] this interval spans about 1.5 Mb of DNA. Long-range cloning in yeast artificial chromosomes [254] (**d**) and cosmids (**e**) now enables systematic gene hunting in this region.


a
11
q
p
b
A-T
S147
THY1
PBGD
S29
APO
S144
S351
DRD2
S132
STMY
S385
S84
1cM
c
A-T
S1647
S1990
S1391
S1300
S927
S1960
S2105
S2003
S2220
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S1819
S1787
S1343
S1817
S2000
S1816
S1325
TEL
CEN
500Kb
FDX
S1897
S535
S384
ACAT
S2022
GRIA4
IL1BC
S611
S1873
d
Y416
Y406
Y402
Y400
Y67
Y16
Y71
Y149
500Kb
e
100Kb

[234] used a rapid method to generate biallelic markers to identify single-strand conformation polymorphisms in DNA fragments isolated from yeast artificial chromosome clones at the A-T region. With these markers they were able to narrow the A-T locus, first to 4 cM between D11S611 and D11S1897 [233] and later to 3 cM between GRIA4 and D11S1897 [234] (fig. 1c). (D11S1897 is a new locus number assigned to a marker formerly included in the locus D11S535.) A physical framework for the A-T region was emerging in parallel from extensive radiation hybrid maps of 11q with particular emphasis on 11q22–23 [235–238] and detailed pulsed-field maps of the A-T region [231, 239]. Integration of the genetic and physical maps [234, 240] narrowed the A-T locus to 3 Mb of DNA. It should be noted that A-T has never recombined with two markers within this interval, D11S384 and D11S535 (fig. 1c).

Genetic analysis of the A-T region has been based up to this point on restriction fragment length polymorphisms and single-strand conformation polymorphism markers. Such markers, usually biallelic, have a limited polymorphic information content and may potentially miss genetic information in noninformative families. Thus, the derivation and mapping of microsatellite markers at the A-T region was considered essential for further narrowing the A-T locus. A high-density microsatellite map of the A-T region was constructed by Vanagaite et al. [241, 242] and Rotman et al. [243, 244] in two stages: microsatellite markers generated at random by several mapping centers and generally mapped to the distal part of 11q were physically mapped within the A-T region [241, 242], and novel markers based on polymorphic CA repeats were generated [243, 244]. A map containing 24 microsatellite markers was constructed across 6 Mb containing and flanking the A-T interval [242] (fig. 1c).

Most of these markers were used in a consortium-based study of 176 A-T families in laboratories in the United States, England and Israel [245, 246]. Lange et al. [246] drew up a comprehensive 20-point linkage map based on the A-T families and 59 CEPH families. Using a Monte Carlo linkage algorithm [247, 248], Lange et al. [246] showed that the peak of the A-T location score was under the marker D11S535, with a 2-lod support interval for A-T between D111819 and D11S1294 (fig. 1c). Haplotype analysis [245, 246] disclosed 4 recombinants which placed A-T distal to D11S1819, and 1 loss of homozygosity in patients from an inbred family, which pointed to an A-T gene proximal to D11S1818. No recombinants were found between A-T and markers in the region D11S384–D11S1294 (fig. 1c). These studies reinforced the notion that the 4 complementation groups in classical A-T are probably determined by mutations at the 11q22–23 A-T locus and may converge to the D11S1818–D11S1819 interval. According to Vanagaite et al. [242] this interval spans about 1.5 Mb (fig. 1c).

Although the evidence for a major A-T locus at D11S1818–D11S1819 is compelling, being based on a significant number of families from various ethnic groups and complementation groups, A-T families have been found that do not link to this region. In the study of Lange et al. [246], 7 out of 176 families did not show linkage to this region: 6 of them had single affected subjects that could represent new mutations, and 1 family had 2 affected individuals who shared the same haplotypes with a normal sibling. In a British A-T family, haplotype analysis in 2 affected cousins and their siblings showed no linkage to 11q22–23 [62]. The patients in this family represented a clinical variant with slower progression of the disease but otherwise had the typical clinical and cellular phenotype of A-T.

Such families may represent still another locus involved in the disease in very rare cases.

Linkage analysis typically reaches its limit of resolution when the disease locus is reduced to 1–2 cM and a final number of recombinants defines its boundaries with no additional recombinants found between them. The locus can be further refined by determining the extent of linkage disequilibrium between the disease and various markers at the locus. A significant degree of allelic association between the disease and a particular marker is usually indicative of physical proximity. The degree of disequilibrium reflects the extent of recombination that has occurred between certain mutations and neighboring markers since these mutations first appeared. This analysis is especially powerful in isolated ethnic communities with a limited number of mutations. A clearly defined core haplotype retained around a particular mutation over the years may help reduce the disease locus [for examples, see 249–251].

Oskato et al. [252] noticed in Moroccan Jewish patients a significant degree of linkage disequilibrium between A-T and a series of markers extending over a wide region, between D11S384 and D11S424 (the latter is distal to D11S1647, fig. 1c). This finding was compatible with a strong founder effect in this community, which has been isolated from the rest of the Jewish people and the surrounding population for many generations. Vanagaite et al. [in preparation] used the microsatellite markers recently added to the map [242] (fig. 1c) and obtained a disequilibrium peak extending between D11S384 and D11S2105 in 16 Moroccan Jewish families. Since several Moroccan Jewish patients have been assigned to complementation group C [81; unpubl. observations], this may indicate that the ATC gene is located at the distal half of the A-T interval. A similar study by Uhrhammer et al. [253] on 27 Costa Rican A-T families showed moderate linkage disequilibrium with several markers in the D11S1816–D11S1300 interval, which did not enable reduction of the disease locus. No significant allelic association between A-T and specific markers was found by Vanagaite et al. [in preparation] in Turkish and Italian families, probably indicating a relatively large number of mutations in these populations. An intriguing allelic association was noticed by Taylor et al. [65] in 10 of 60 British A-T families in which the patients have a clinical variant of A-T, with later onset and lower chromosomal radiosensitivity than most of A-T patients. One of the chromosomes in each of these patients has a specific haplotype at the D11S1819–D11S1817 region, extending proximal to the A-T locus defined by linkage analysis (fig. 1c). It is not clear yet whether this finding flags a gene involved in the disease in a subset of patients who might harbor a mutation with a milder phenotypic effect.

In the course of these studies, as the boundaries of the A-T locus moved closer together, systematic cloning of the region in genomic contigs became essential. Rotman et al. [254, 255] constructed a yeast artificial chromosome contig encompassing the current A-T locus (fig. 1d), and similar contigs have been constructed in other laboratories involved in positional cloning of the A-T gene(s) [James, MR; McConville, CM; Gatti, RA; Concannon, P, pers. commun.]. Cosmid contigs encompassing various portions of the current A-T interval have been constructed in several laboratories (see fig. 1e for example) and used in the next step in the positional cloning scheme, gene hunting.

Gene hunting typically relies on two commonly used methods to identify transcribed sequences in genomic DNA: direct selection based on hybridization of genomic DNA with cDNA collections of various sources [256, 257] and exon trapping which identifies and clones

exon sequences by virtue of their splicing capacity [258]. Transcription maps of the A-T locus indicate that this genomic region is gene rich and contains a high density of transcribed sequences, most of which are new [259; Bar-Shira et al., in preparation]. This wealth of genes within the A-T locus makes the search for mutations in patients an experimental challenge.

Conclusion

Decades of intensive research have provided numerous clues to the nature of the defect in A-T, but this defect might not be delineated at the molecular level before the culprit gene(s) are cloned. Until then, the disease remains largely a mystery, as it has been 'from the start' [1]. Localization of the A-T locus to chromosome 11q22–23 and subsequent application of positional cloning have, however, brought A-T researchers closer than ever to unraveling this mystery.

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