

Meeting Report

Fourth International Workshop on Ataxia-Telangiectasia¹

Ataxia-telangiectasia is a multisystem single gene disorder with defects in the neurological, vascular, and immune systems. Affected individuals deteriorate progressively, and they are highly cancer prone. The patients, as well as cells cultured from them, are hypersensitive to the lethal effects of ionizing radiation, suggesting that A-T² cells may be deficient in the ability to recognize, repair, or process radiation damage. Elucidation of the molecular basis of the primary defect in A-T is thus of great interest for a wide variety of disciplines. The fourth international A-T meeting was organized by Dr. R. A. Gatti (University of California, Los Angeles, CA), with the emphasis on the localization of the A-T genes. (For proceedings of the previous meetings, see Refs. 1-3.)

In an introductory review, Dr. E. Boder (University of California, Los Angeles, CA), who has studied A-T patients extensively over the past 30 years, gave a survey of her recent clinical observations. She suggested that many of the clinical features could be regarded as manifestations of premature aging, and she emphasized that modern therapeutic treatments can now alleviate some of the symptoms of the disease.

Genetic studies presented by N. Jaspers (Erasmus University, Rotterdam, The Netherlands) have identified four distinct complementation groups, named A, C, D, and E, as well as two further groups (V1 and V2) of the closely related but clinically distinct Nijmegen breakage syndrome described by C. Weemaes (University Hospital, Nijmegen, The Netherlands). The current assignment of 41 A-T and 9 Nijmegen breakage syndrome fibroblast strains from 44 families to these groups has been published recently (4). Eighty % of assigned patients fall into groups A and C.

A great impetus to the search for the A-T genes was provided by Gatti and his colleagues, who localized the AT-A gene to chromosome 11q22-23 by linkage studies of 170 restriction fragment length polymorphisms in a series of A-T families (5). Several groups described their involvement in the fine structure mapping of this region either by linkage studies [M. Lathrop (Centre d'Etudes des Polymorphismes Humaines, Paris, France), P. Charmley (University of California, Los Angeles, CA), P. Concannon (Virginia Mason Research Center, Seattle, WA)] or by using chromosome breakpoints occurring in rodent/human cell hybrids [L. Doucette-Stamm (MIT, Cambridge, MA)] or tumor cells [J. Kersey (University of Minnesota, Minneapolis, MN)]. There was general agreement on the map positions of the various markers shown in Fig. 1. The possible use of other markers that map near the 11q22 band, such as the APO-A1/C3/A4 complex described by S. Karathanasis (Harvard, Cambridge, MA) or the dopamine receptor described by O. Civelli and M. Litt (Oregon Health Sciences, Portland, OR), was also discussed. Saturation mapping of the 11q chromosome arm with contiguous cosmid clones, together with analysis by pulse-field gel electrophoresis and rare-cutting restriction enzymes as carried out by G. Evans (Salk Institute, San Diego, CA), will prove of immense value in providing a complete physical map of the area.

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² The abbreviation used is: A-T, ataxia-telangiectasia.

In order to locate the gene more precisely it is necessary both to obtain an accurate and detailed map of the region (see above) and to study as many A-T families as possible. Gatti reported that extension of his previous study (5), using additional markers on more families, suggests that the most likely position for the AT-A gene is between pYNB3.12 and stromelysin, as indicated in Fig. 1. Studies reported by C. McConville and A. M. R. Taylor (Birmingham University, Birmingham, United Kingdom) on 35 independent A-T families were consistent with this location, but an alternative position telomeric to Thy-1 was also consistent with their data. The chromosomal localization of AT-A was further supported by linkage studies on additional A-T families, as discussed by Y. Shiloh (Tel Aviv University, Ramat-Aviv, Israel) and M. Swift (University of North Carolina, Chapel Hill, NC). Shiloh also provided preliminary evidence for linkage of the AT-C gene to the same region as AT-A. Further progress in fine structure mapping will depend on the identification of more genetically characterized A-T families and the finding of additional polymorphisms to increase the informativeness of these families, as discussed by W. Salser (University of California, Los Angeles, CA).

An alternative method for determining the chromosome localization of A-T genes was described by E. C. Friedberg and C. Lambert (Stanford University, Stanford, CA). This was based on correction of the radiosensitivity of A-T fibroblasts by the normal human gene. Microcells prepared from mouse cell hybrids containing a small number of human chromosomes, one of which was "tagged" with a dominant marker gene, were fused to A-T cells. Selection was applied for those cells which had incorporated the tagged chromosome and the radiosensitivity of the resulting cells was measured. Cells which had recovered normal radioresistance were assumed to have been corrected by the incorporated chromosome, which could then be identified. Using this procedure they presented evidence to show that a gene on chromosome 22 corrected AT-D cells.

Direct procedures to correct the defect in A-T and other human cells by DNA-mediated gene transfer have not been successful because, as is now apparent, SV40-transformed human fibroblasts, unlike some rodent cell lines, can stably maintain only very small amounts of exogenous DNA in their chromosomes, according to Jaspers and A. R. Lehmann (Sussex University, Brighton, United Kingdom). Possible future approaches to isolate human radiosensitivity (DNA repair) genes such as A-T by gene transfer procedures include the use of extrachromosomally replicating Epstein-Barr virus vectors, described by M. R. James (Centre National de Recherche Scientifique, Paris, France) and the cloning of human genes correcting mutant rodent cell lines (Jaspers) or mutant fission yeast strains (Lehmann) with phenotypes resembling that of A-T.

Although A-T (homozygote) patients represent a very small class of highly cancer-prone individuals, the heterozygote carriers comprise about 2% of the human population, and extensive retrospective studies of Swift *et al.* (6) of A-T families had already indicated a 6-fold increased risk of breast cancer in A-T heterozygotes. Swift reported on his first results from a prospective study of more than 200 A-T families. These indicated an 8.5-fold increased relative rate ratio of breast cancer and a 2.6-fold (male) and 4-fold (female) increased relative rate

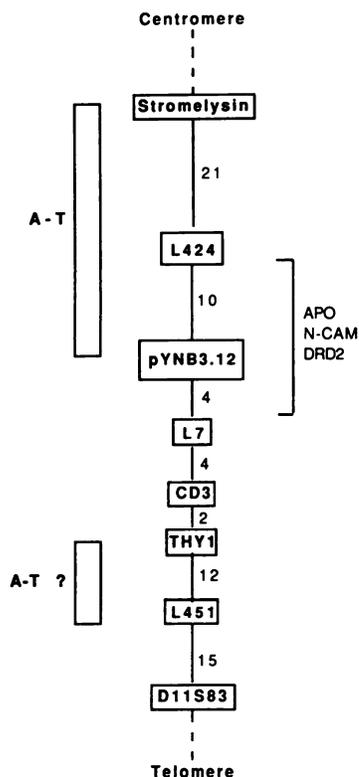


Fig. 1. A working map of the chromosome 11q22-23 region. Localized genes and restriction fragment length polymorphisms are shown in boxes with approximate distances in centimorgans between them. The best estimates for localization of three further genes are indicated at the right and of the AT-A gene at the left. Note the following alternative designations: L424, D11S132; pYNB3.12, MCT128.1, D11S148, D11S144, D11S285; L7, D11S29; L451, R548, D11S133, D11S138.

ratios of all cancers in A-T blood relatives. These data suggest that as many as 20% of all breast cancers may be associated with A-T heterozygosity. A reliable procedure for identifying A-T heterozygotes would therefore be of immense value. As a group A-T heterozygotes are known to be more radiosensitive than normal individuals. However, tests for the detection of heterozygotes based on this property suffer from a considerable overlap with the normal range, when using as an end point either cell killing, as discussed by M. C. Paterson (Cross Cancer Institute, Edmonton, Alberta, Canada), or the occurrence of micronuclei in exfoliated cells of buccal smears, described by M. Rosin (Cancer Research Center, Vancouver, British Columbia, Canada). K. Sanford (National Cancer Institute, Bethesda, MD) has measured chromosomal radiosensitivity in G_2 fibroblasts and identified patients with a variety of cancer-prone disorders, including A-T heterozygotes, by increased sensitivity in this test. In a blind study of 30 samples from A-T families she succeeded in identifying all 10 obligate heterozygotes. However, both this test and that of Paterson failed to detect other heterozygotes identified by linkage studies in AT-A and AT-C families, as reported by Shiloh and D. Weeks (Columbia University, New York, NY). A possibly important implication for radiotherapy of the identification of A-T heterozygotes was discussed by A. Norman (University of California, Los Angeles, CA). He pointed out that 5% or more of breast cancer patients were unusually radiosensitive. If these patients could be screened out prior to treatment, the remaining patients could tolerate higher radiation doses, thus improving therapeutic efficiency. These radiosensitive breast cancer patients might be A-T heterozygotes, so that a detection procedure for heterozygotes could improve therapy treatment not only for them but

also for cancer patients in general.

Two interesting new cellular abnormalities in A-T were described by M. Lavin (Queensland Institute for Medical Research, Brisbane, Australia) and S. Meyn (Yale University, New Haven, CT). Using gel shift assays Lavin identified a protein in extracts of A-T cells which specifically bound to a fragment of DNA containing a promoter/enhancer region. This protein was not present in extracts of normal cells unless they had been γ -irradiated. The radiation-stimulated appearance of this protein in normal cells was not inhibited by cycloheximide and therefore presumably represented a structural alteration rather than induction of a new protein. Meyn devised an elegant retroviral system for measuring the rate of somatic recombination in cultured cells. His preliminary results suggested that the recombination frequency in an A-T cell line was 100-fold elevated over that of a xeroderma pigmentosum or a normal cell line.

The meeting closed with a presentation by N. Lench (St. Mary's Hospital, London, United Kingdom) on attempts to isolate the gene for cystic fibrosis. The sobering message from this work was that even though chromosomal localization represents a major advance toward cloning a gene, the subsequent pinpointing and cloning of the gene itself are still formidable tasks. A-T, however, offers a major advantage over genes such as those for cystic fibrosis, Huntington's disease, or muscular dystrophy in that a functional assay exists for the A-T genes. The A-T genes should be able to restore normal radiation resistance to A-T cells from the appropriate complementation groups. The collaborative atmosphere at the meeting and willingness to exchange cell lines, probes, and linkage data should help toward cloning and characterizing the A-T genes and increase our understanding of this fascinating cancer-prone disorder.

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