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Meeting Report

Sixth International Workshop on Ataxia-Telangiectasia[1]

In patients with A-T,[2] there is a 70–250-fold excess of lymphocytic leukemias and lymphomas, mostly occurring in childhood. There is also a smaller increase in the frequency of epithelial cell tumors. In addition to this tumor phenotype associated with the *A-T* gene in the homozygous state, there is the possibility that carriers of the gene might also be at an increased risk of developing some tumors. One of the major goals of A-T research today is to try to explain the nature of this predisposition to both lymphoid and epithelial cell tumors in patients. Some might suggest that the latter goal will only be realized following the cloning of the gene. The majority of contributions presented at ATW6 concerned one or another of these areas of research.

The clinical features of ataxia-telangiectasia, as reviewed by Bundey, are first noted in infancy and result from abnormalities in a variety of organ systems including the cerebellum, immune system, liver, and gonads. The cerebellar degeneration leads to a severe progressive ataxia, dysarthria and oculomotor apraxia. A-T patients are unusually sensitive to the effects of ionizing radiation; this can be observed at the cellular level as well.

In his introductory abstract for the ATW6 workshop, Harnden writes, "It is now almost 70 years since the first description in the medical literature of a syndrome recognisable as ataxia-telangiectasia, about 30 years since the association of A-T with malignant disease and 20 since the recognition of radiosensitivity at both patient and cellular level." To this could be added, "and five years since an *A-T* gene was localized to chromosome 11q22–23." "Yet," continues Harnden, "we still know remarkable little about the origin or pathogenesis of this disease."

Each of the previous six workshops has painstakingly brought us a little closer. In 1981 (ATW1), a better phenotype was described. In 1985 (ATW2), the neurodegenerative nature of A-T was clarified and plans for a "genetic attack" were developed. By 1987 (ATW3), as part of that attack, many key families around the world had been assigned to complementation groups, primarily through the work of Jaspers and coworkers. In 1989 (ATW4), formal presentations and confirmations were made regarding the localization of an *A-T* gene to chromosome 11 (1). This was the beginning of positional cloning. A higher resolution map of distal chromosome 11 was already under way, taking advantage of the burgeoning new genome projects around the world, such as the CEPH linkage mapping consortium in Paris. However, at that time, most investigators believed that the *A-T* gene would be cloned not by the slower positional cloning approach but by more direct transfection and complementation experiments. Indeed, the candidate *A-T* gene (*ATDC*) isolated by this method was much discussed at ATW5 (1992). However, it was already becoming clear that complementational cloning was potentially misleading. The ATW6 workshop confirmed this, and most investigators admitted that they had largely abandoned this approach after having cloned many complementing (or partially complementing) genes by this method that did not map to chromosome 11. An informal poll of attendees by Meyn identified over 20 human cDNAs that complement A-T radiosensitivity. The conclusion was that complementation studies are best reserved for confirmation of a candidate gene that maps to the region defined by positional cloning.

Mapping *A-T* Genes Using Recombination Events and Haplotypes

Positional cloning now localizes the A-T region to less than 1 megabase, between the genetic markers S1818(A2) and S1819(A4) (Fig. 1); this represents primarily the collaborative linkage analysis efforts of workers in the laboratories of Gatti (1–4), Taylor (5–7), and Shiloh (8), encompassing over 175 families. Within this region, Swift corroborated finding no recombinants with S384(CJ52.193) in 42 informative families and 1 recombinant proximal to S1897(Y12.8). McConville also showed two haplotypes with ancestral recombinations that place A-T proximal to S535(J12.8), further narrowing the target region. James and Rotman presented physical maps of the "A-T region." Taken together, these data allow the "last step" of positional cloning to begin, *i.e.*, the isolation and characterization of transcripts from within this region. These experiments are already under way, promising that the *A-T* gene will be identified very soon. Sankhavram gave a very practical overview of ways to optimize conditions for "cDNA selection" and discussed some of the pitfalls as well.

Many participants at the workshop asked, "How many *A-T* genes are there, and do they all localize to 11q22–23?" Gatti presented an update of the haplotyping on the Amish family that was used originally to localize the group A gene to chromosome 11, showing now that recombinants localize the group A gene (*ATA*) to between S1343 and S1300 (Fig. 1). A group C Israeli pedigree originally described by Ziv *et al.* (8) localizes the *ATC* gene distal to S1819(A4); no recombinants have yet been observed that would define this region distally. Oskato *et al.* (9), however, recently described haplotypes among Moroccan Jews that both overlap the ATA candidate region and possibly extend the ATC region distally to CJ77(S424); their highest association scores being between S1818(A2) and S927, according to Rotman. Group D fibroblasts are complemented by chromosome 11 (10). A single group E family is compatible with linkage to 11q22–23, sharing one haplotype with other UK families; one other Australian group E family remains for study. Edwards spoke about the Extract Haplotypes series of computer programs and used the United Kingdom A-T haplotype database to demonstrate their potential effectiveness.

Taylor described an interesting subset of 10 A-T families in whom the radiosensitivity of affecteds is intermediate between normal and typical A-T levels, and the onset or progression of symptoms is delayed. Within this group, a unique shared genetic haplotype was identified in 8 of 10 families [S1817(allele 4), S1343(allele 1), and S1819(allele 8)]; 5 of the 8 families were of Scottish/English Midlands origin.

NBS and A-T

The relationship of NBS to A-T was further clarified at the workshop by Weemaes. NBS patients have neither ataxia nor telangiectasia but share many other characteristics with A-T, including radiosensitivity, characteristic chromosomal aberrations, immunodeficiency, and cancer susceptibility. Most families are of Eastern European origins. In 1988, Mexican twins were described by Curry *et al.* (11)

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[1] The workshop was organized by Drs. A. M. R. Taylor, D. Scott, C. F. Arlett, and J. Cole, and was held at the University of Birmingham, United Kingdom from March 22–25, 1994. It was sponsored by the A-T Society, the A-T Children's Project, the Medical Research Council, the British Association for Cancer Research, the United Kindom Coordinating Committee on Cancer Research, the A-T Medical Research Trust, the A-T Research and Support Trust, the Cancer Research Campaign, and the Wellcome Trust.

[2] The abbreviations used are: A-T, ataxia-telangiectasia; ATW6, the Sixth International Workshop on Ataxia-Telangiectasia; cDNA, complementary DNA; NBS, Nijmegen Breakage Syndrome; PKC, protein kinase C; RDS, radioresistant DNA synthesis; TCR, T-cell receptor; LCL, lymphoblastoid cell lines.

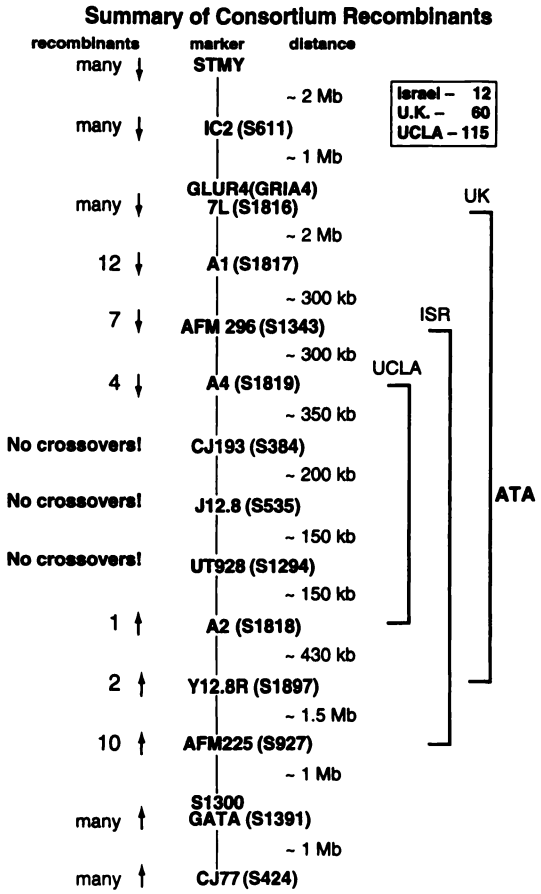

Fig. 1. Summary of observed recombinants. The distances shown were derived from both linkage analyses of CEPH and A-T families, cloned segments, and pulsed field gel mapping. The number and ethnic origin of families on which the brackets are based is shown in the box (upper right).

who had both A-T and NBS and were called AT_{Fresno}; their fibroblasts complemented all A-T groups except V1 (variant 1 of NBS). This suggested that the same defective gene caused both NBS-V1 and A-T_{Fresno}. Gatti presented data on two AT_{Fresno} families that support the probability of linkage to 11q22–23. Linkage studies on V1 families are still in progress. Gatti also presented a linkage analysis of a NBS-V2 (radiosensitive) family that suggested nonlinkage to chromosome 11q22–23. This was corroborated by a report by Komatsu on the failure to complement several V2 fibroblast lines with chromosome 11. V1 studies are also in progress in Komatsu's laboratory. Taylor presented haplotype data on an English NBS family that did not link to chromosome 11; it was not known whether this family represented V1 or V2.

Thus, all *A-T* genes but V2 (and possibly V1) appear to localize to chromosome 11q, probably to 11q22–23. In such a model of multiple genes mapping to a single small region of the genome, it is probable that they would be evolutionarily related and possibly share homology. They might even code for subunits of a single protein complex. It is, of course, still possible that only a single *A-T* gene exists on chromosome 11, perhaps coding for a protein that is alternatively spliced, with mutations in different gene segments accounting for the complementation phenomena.

The ATD-complementing Gene

Is *ATDC* an *A-T* gene? Four followup reports on the ATD-complementing (*ATDC*) gene, a gene described in 1992 (12) that complements a group D fibroblast line, were given by Kapp, Hosoi, Murnane, and Christman. Kapp indicated that *ATDC* also complements group A and group C fibroblasts. Using a yeast two-hybrid system, Christman found that the *ATDC* product binds to a number of different proteins, including an inhibitor of PKC, vimentin, and a brain-specific protein. Using nickel-agarose columns, Christman confirmed the binding to vimentin. *ATDC* is alternately spliced and is expressed in placenta, thymus, prostate, colon, fetal lung, and fetal kidney. However, no mutations were found in the sequencing of the 3-kilobase cDNA product from one group D A-T patient, and in single-strand conformational polymorphism analyses of the cDNA from another group D patient. This gene is interesting, nonetheless, in that it contains a leucine zipper and two zinc finger domains. It appears to be a member of the *APL/PML* leukemia gene family, and probably functions as a DNA transcription factor. The fact that it functions somewhere in the cell cycle, upstream from p53, may sustain some interest in it as a candidate *A-T* gene. However, it has no effect on RDS, and linkage evidence is lacking for the localization of any *A-T* gene outside the 11q22–23 region (except for NBS-V2 and possibly NBS-V1, but NBS families have been traditionally excluded from all linkage analysis computations). The *ATDC* gene maps approximately 30,000,000 base pairs distal to the A-T region, as defined by positional cloning.

Genomic Instability

The workshop brought together, more clearly than has been done before, the many cellular features of A-T. The fundamental defect appears to involve either a defect in DNA processing or a defect in a DNA damage surveilllance network.

Kirsch, Thick, and Russo discussed a complex groups of observations regarding the chromosomal breakpoints of T-cell leukemias in A-T, as well as in cytogenetically similar leukemias in non-A-T patients. Kirsch reviewed molecular evidence showing that T cells from A-T patients had 10–100-fold higher levels of TCR interlocus rearrangements than normal. Lymphoid tumors in A-T patients, however, appear to arise in cells with translocations involving only one *TCR* gene. Emphasis was given to two types of rearrangement: (*a*) Russo discussed inv(14) inversions or t(14,14) translocations involving the TCR Jα region and TCL1, the latter involving additional rearrangements as well; and (*b*) Thick described patients with t(X;14) translocations involving, again, TCR Jα, and the genes *C6.1A* or *C6.1B* on Xq28. All the evidence suggests that it is the *C6.1B* gene that plays a role in the initial clonal expansion of T cells with the t(X;14) translocation. From the data on interlocus recombination, Kirsch speculated that A-T may be a disorder of structural or temporal chromatin accessibility.

Thacker, Cole, Hittleman, and Bryant attempted to further define the A-T defect. Human cell extracts were used to foster rejoining of DNA following double strand breakage of a plasmid by different restriction enzymes. Thacker showed that extracts from some A-T cell lines caused misrejoining of some break sites more frequently than extracts from normal cells, and this misrejoining involved deletions of 20–600 base pairs that occurred between short direct repeats (nonconservative recombination), a mechanism not unlike Meyn's spontaneous intrachromosomal recombination (13). Thacker has proposed a strand-exposure and repair model for this recombination process whereby "single strand exposure (by nuclease or helicase) is followed by annealing at the direct repeats, removal of single strand tails, and repair of the gapped duplex." Meyn suggested that these may correspond to topoisomerase II sites.

Using premature chromosome condensation, Hittleman noted that the yield of double strand breaks in A-T cells is normal; however, the frequency of chromosome damage is increased, suggesting that the

A-T defect causes a high rate of conversion of double strand breaks into chromatid aberrations. He suggested that the reason for this was that in A-T cells a higher fraction of DNA is found in the "intermatrix" region which would allow adjacent matrix clusters to fall apart (aberrations), rather than being held together on a nuclear matrix. In this case, the DNA would be more accessible simply by virtue of its altered structure.

Bryant arrived at similar conclusions; he used restriction endonucleases to induce double strand breaks, measuring the efficiency of rejoining in A-T *versus* normal cells by neutral filter elution. His studies showed that blunt-ended (*Pvu*II-induced) breaks caused more damage in A-T cells than cohesive 5′ (*Bam*HI-induced) or 3′ (*Pst*I-induced) ends. It appeared that although double strand breaks are not processed efficiently by A-T cells, no specific defective repair mechanism can be identified. Nor is it clear what causes double strand breaks *in vivo* in A-T patients, an area that merits further attention since it could also provide a therapeutic opportunity.

Cell Cycle Checkpoints and p53

Attempts were made to evaluate and possibly link three features of A-T cells: (*a*) increased cellular radiosensitivity; (*b*)p53 induction; and (*c*) *in vitro* apoptosis. In 1992, Kastan *et al.* (14) reported that, unlike in normal cells, there is no increase in p53 expression in A-T cells following their exposure to ionizing radiation. This issue received a great deal of attention at the workshop, including an overview of radiation checkpoint model systems by Carr. Lu could not convincingly confirm Kastan's observation. Using fibroblasts from 10 different A-T individuals, she observed only a delay in p53 expression in A-T cells when tested 6 h after radiation. Lavin showed that the p53 response is reduced and/or delayed in A-T LCLs from complementation groups A, C, D, and E. He also provided evidence suggesting that the product of the p53 effector gene, *WAF1(p21)*, is defective as well in the radiation response of A-T cells, confirming a defect in the p53 pathway. In similar work, Hall observed that two LCLs expressed EBNA5, which interacts with p53. She also noted a normal p53 response in two LCLs derived from breast cancer patients (both from families linking to chromosome 17).

When Lavin used other agents to block cell cycle progression, those that do not break DNA, the p53 response of A-T cells was normal. The p53 response was also normal following UV irradiation. Lavin next considered the role of PKC and other signal transducers in the pathway upstream from p53, a likely place for an A-T protein to function (15). Direct measurements of $PKC_{epsilon}$ were markedly decreased in group C and group D A-T LCLs. Lavin concluded that although the $PKC_{epsilon}$ isoform, 1 of about 14 such isoforms, is probably not the A-T protein, other PKC isoforms and the molecules with which they interact deserve further scrutiny.

Meyn presented evidence for a Damage Surveillance Network model in which no specific defect in DNA repair would exist, but the lack of a functional *A-T* gene would result in both an inability to halt the cell cycle at checkpoints and an unusually low threshold for triggering p53-mediated programmed cell death (apoptosis) by spontaneous and induced DNA damage. Beginning at 24–48 h after radiation or exposure to a radiomimetic mutagen (streptonigrin), apoptosis was detectable in all four A-T fibroblast lines but not in normal cells. In normal cells, disruption of the p53-mediated G_1-S cell cycle checkpoint alone results in an increased rate of spontaneous intrachromosomal recombination but does not result in radiosensitivity; therefore, the loss of the p53-mediated G_1-S checkpoint observed in A-T cells does not explain their radiosensitivity. However, when both the *p53* and *A-T* genes were defective (by transfection of a dominant-negative *p53* mutant into A-T fibroblasts), Meyn observed a normalization of radiosensitivity as measured by cell survival. UV irradiation did not induce apoptosis.

Reports by Arrand, Lohrer, Zdzienicka, and Jeggo, using either cell fusion or microcell mediated chromosome transfer to correct radiosensitive rodent mutants resembling A-T, reiterated the efficiency with which new radiation repair genes can be cloned by such approaches. While none of these studies has as yet yielded an *A-T* gene, genes that correct various responses to radiation damage have been identified on human chromosomes 2, 5, 8, and others. Jeggo *et al.* (16) mapped a fragment of human DNA that corrected the radiosensitivity of the *xrs-6* mutant to chromosome 2q33–35. This map position corresponded to that of *Ku80*, a gene with end-binding activity (17). Constructs of *Ku80* complemented the V(D)J recombination defect, the radiophenotype of *xrs-6*, and restored the end-binding activity. Jeggo's experience with *Ku80* also exposed the disturbing fact that of five YAC clones used in her original studies, none, in retrospect, contained an intact *Ku80* gene.

A-T Heterozygotes and Cancer

Scott updated progress on a "G_2 radiosensitivity assay" for identifying A-T heterozygotes, which is based on the observation that cells are damaged and repaired in a different way than when irradiated in G_1 or S phase. Further, when cells from A-T heterozygotes are irradiated in G_2, they show higher levels of chromosome damage than controls. Both a previous National Cancer Institute study (18–19) and his own "Paterson" study (*i.e.*, performed at the Paterson Institute), however, do show some overlap between controls and A-T heterozygotes. It now appears that this assay may be identifying individuals who are both radiosensitive and cancer susceptible as a result of defects at several genes. These may be genes that play a role in cell cycle checkpoints, such as the cyclin kinases, WAF1(p21), Rb, and MTS1(p16) (CD4K-inhibitor) (20–21). Scott also found increased G_2 radiosensitivity in about 40% of breast cancer patients, both before and after treatment, a figure much higher than the predicted frequency of A-T heterozygotes among such patients.

Easton presented an update on the incidence of breast and other cancers in parents and grandparents of British A-T patients. In this population, the estimated relative risk for breast cancer in all A-T heterozygotes is 1.3. The relative risk estimate for breast cancer based on parents alone is 6.3. With the use of a gene frequency of 0.5% and a relative risk of breast cancer of 3.9 (using all currently available data), an estimated 3.8% of breast cancer cases would be attributable to the *A-T* gene. Is the *A-T* gene an important component in the known familial clustering of breast cancer? Linkage studies by Wooster *et al.* (22) and Cortessis *et al.* (23) have thus far failed to find linkage to A-T region markers in breast cancer families. Whether this result conflicts with epidemiological results suggesting an increased risk of breast cancer in A-T heterozygotes remains unclear.

Borresen and Chessa also reported an increased frequency of breast cancer in A-T heterozygotes from Norway and Italy, respectively. Chessa further noted an increased incidence of gastric carcinoma in grandparents of A-T affected patients, a finding previously noted by Porras *et al.* (24) in Costa Rican A-T families. However, Arlett commented that gastric carcinoma has not been noted in United Kingdom families. Borresen, using three highly informative markers from the "A-T region," reported loss of heterozygosity in one breast cancer and one colon cancer from A-T heterozygotes. In addition, in one breast cancer specimen from an A-T homozygote, she noted new alleles, suggesting genomic instability in A-T similar to that noted in nonpolyposis colon cancer (25). While this important observation needs to be confirmed and extended, it suggests that the *A-T* gene could be a mismatch repair defect, similar to those seen in yeast mutants. However, human homologues of three known mismatch

repair genes in yeast have already been mapped to chromosomes other than 11.

Prenatal Diagnosis

Recent experiences with prenatal testing were reviewed by Jaspers, Woods, Gatti, Varma, and McGuire. Jaspers contends that RDS on cultured fetal cells from either chorionic villi or amniotic fluid is a reliable means of functionally evaluating the *A-T* gene(s). He recommends checking the prior affected (index) child as well so as to confirm the diagnosis of A-T in each family. Gatti and McGuire reviewed genetic haplotyping results, the accuracy of which was also good, as shown by followup studies. However, haplotyping for A-T is based on an assumption of linkage to chromosome 11q22–23. Gatti and Taylor reported that to date, 5 of about 175 families do not "appear" to link. Although these families are still under study, all but one of the nonlinking families they described contained only one affected sibling, suggesting that these may represent spontaneous or new mutations rather than linkage to an *A-T* gene on another chromosome. Nevertheless, genetic haplotyping in families with spontaneous mutations could still result in prenatal misdiagnoses. Further, two variant families that were not radiosensitive by colony survival assay (26) did not link to chromosome 11. Thus, although prenatal diagnosis by haplotyping alone probably is accurate in more than 98% of families, the consensus opinion was that both RDS and haplotyping should be used until an *A-T* gene sequence is available for direct analyses. It was further recommended that such studies only be undertaken by laboratories with long-standing experience in clinical laboratory testing.

Conclusion

After hearing 45 reports presented over 2½ days, it appeared that 3 lines of investigation were converging quickly on the isolation of the *A-T* gene (or genes): (*a*) positional cloning; (*b*) complementational cloning (using cell fusions and chromosome transfer); and (*c*) cell cycle biochemistry/radiobiology. A short discussion of gene therapy was judged premature, but most participants expected that ATW7 will be focused squarely on this issue, assuming that an *A-T* gene will have been isolated by then.

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