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Determining the Role of the Telomere on
the Severity of Birth Defects

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Determining the Role of the Telomere on the Severity of Birth Defects

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Abstract

This is the final report of a three-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). The work resulted in publication of eight manuscripts in peer-reviewed journals. Ten papers were presented at meetings of professional societies and one of these resulted in an award to a student for "Outstanding Undergraduate Research." The research conducted under this program has had a profound effect on the characterization of a human malady, the Wolf Hirschhorn syndrome (WHS), caused by a terminal deletion of chromosome 4. The available genomic sequence and our attempts to characterize the potential transcriptional units has sharpened our focus on the development of methods to explore the functional significance of the human genome.

Background and Research Objectives

There are a variety of birth defects associated with the loss of genetic material near the ends of chromosomes. At least one-third of human chromosomes can have terminal deletions that result in live births but lead to significant clinical abnormalities.

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Terminal deletions can arise by a variety of mechanisms, including the clastogenic effects of ionizing radiation. It is clear that chromosome stability is severely impaired if the terminal structure, the telomere, is missing. In fact, complete chromosomal loss (a condition incompatible with life in humans) has been observed in experimental organisms when the telomere is removed or disrupted. The telomere is a unique chromosomal element that has been demonstrated to generate novel structures, to initiate DNA replication at the ends of chromosomes, and to affect the expression of genes in its proximity.

The goal of this project was to evaluate the effect of the telomere on gene expression in human cells derived from patients with a birth defect resulting from a terminal deletion. We have utilized cell lines derived from patients with a serious birth defect called the Wolf Hirschhorn syndrome (WHS). One of the hallmark diagnostic criteria for WHS is the deletion of the terminal segment of human chromosome 4.

These patients are referred to as $4p^-$. In the course of these studies we have developed a series of landmark cosmid probes, currently being employed by researchers around the world, to evaluate suspected WHS patients. Cosmids are relatively small segments (45,000 bp) of DNA derived primarily from the human genome but capable of being propagated in bacteria to generate sufficient mass for subsequent studies. Random cosmid clones organized into arrays of DNA segments representing their position in the genome are referred to as contigs. Furthermore, we have identified a series of novel genes, characterized their pattern of expression, and generated a variety of reagents to further these studies. While the product of gene expression is RNA, they are represented by cDNA (complementary DNA) clones derived from cellular RNA.

In an effort to define genes whose expression might be altered by a change in telomere position, we utilized cell lines to determine the smallest region of overlap, which would, therefore, define the critical region (summarized by the patient cell lines represented in Figure 1b). The Wolf Hirschhorn syndrome critical region (WHSCR) is defined by this smallest region of overlap and is likely to contain genes critical to normal human development. Cell lines derived from WHS patients were analyzed using the technique of fluorescent in situ hybridization (FISH) to define the smallest region of overlap. A small group of cosmids, the landmark set, were used in the primary analysis. Once the approximate breakpoint was defined, the specific genomic segment containing the deletion breakpoint can be identified using the individual members of the contig between the landmark clones (called second tier cosmids). Genes identified in this segment are being studied using a variety of molecular genetic techniques to determine their physiological role

in human biology. Finally, we hope that these studies will then allow us to evaluate how the alteration in telomeric position modulates their expression.

Scientific Approach and Accomplishments

The research conducted under this program has had a profound effect on the characterization of a human malady, the Wolf Hirschhorn syndrome (WHS), caused by a terminal deletion of chromosome 4. The specific details of this work is contained in the manuscripts listed in the Publications section below.

In summary, our efforts resulted in the generation of a series of "landmark" cosmid DNA clones that are being employed by researchers around the world to characterize WHS patients with a uniform set of reagents. This has had a direct impact on genetic characterization of the disorder. We have identified approximately 15 putative coding sequences that might contribute to the WHS. This effort was facilitated by the availability of complete genomic sequence available for the entire WHS critical region. The entire genomic sequence of this region was analyzed by BLAST. As a result several independent cDNA clones were identified from the dbEST data base. In addition, computer algorithms designed to detect potential coding sequences have detected several other candidate genes. These cDNAs show no significant similarity to members of other DNA or protein databases. Furthermore, these genes have been localized within the WHS critical region and reveal an interesting pattern of transcriptional organization. The available genomic sequence and our attempts to characterize the potential transcriptional units has sharpened our focus on the development of methods to explore the functional significance of the human genome.

Publications

Peer Reviewed Manuscripts Resulting from LDRD Support

Altherr, M.R., T.J. Wright, K. Denison, A. V. Perez-Castro and V.P. Johnson. 1997. Delimiting the Wolf-Hirschhorn syndrome critical region to 750 kilobase pairs. *Amer. J. Med. Genet.* 71:47-53.

Fang, Y.Y., S. Bain, E.A. Hann, H.J. Eyre, M. MacDonald, T.J. Wright, M.R. Altherr, O. Reiss, G. Sutherland and D.F. Callen. 1997. High Resolution characterization of an interstitial deletion of less than 1.9 Mb at 4p16.3 associated with Wolf Hirschhorn syndrome. *Amer. J. Med. Genet.* 71:453-457.

Perez-Castro, A.V., J. Wilson and M.R. Altherr. 1997. Genomic organization of the human fibroblast growth factor receptor 3 (*FGFR3*) gene and comparative sequence analysis with the mouse *Fgfr3* gene. *Genomics* 41:10-16.

Wright, T.J., D.O. Rieke, K. Denison, S. Abmayr, P.D. Cotter, K. Hirschhorn, M. Keinänen, D. McDonald-McGinn, M. Somer, N. Spinner, T. Yang-Feng, E. Zackai and M.R. Altherr. 1997. A transcript map of the newly defined 165 kb Wolf-Hirschhorn syndrome critical region. *Hum. Molec. Genet.* 6:317-324.

Wright, T.J., M. Clemens, O. Quarrel and M.R. Altherr. Wolf-Hirschhorn and Pitt-Rogers-Danks syndromes caused by overlapping 4p deletions. *Amer. J. Med. Genet.*, in press.

El-Rifai, W., J. Leisti, M. Kahkonen, A. Pietarinen, M.R. Altherr and S. Knuutila. 1995. A patient with Wolf-Hirschhorn syndrome originating from translocation t(4;8)(p16.3;q24.3)pat. *J. Med. Genet.* 32:65-67.

Perez-Castro, A.V., J. Wilson and M.R. Altherr. 1995. Genomic organization of the mouse fibroblast growth factor receptor 3 (*Fgfr3*) gene. *Genomics* 30:157-162.

Johnson, V.P., M.R. Altherr, J.M. Blake, L.D. Keppen. 1994. FISH detection of Wolf-Hirschhorn syndrome: exclusion of D4F26 as critical site. *Am. J. Med. Genet.* 52:70-74.

Papers Presented at Meetings of Professional Societies Resulting from LDRD Support

Perez-Castro, A.V., J. Wilson and M.R. Altherr. 1997. Comparative genomic sequencing of the human and mouse fibroblast growth factor receptor type III gene. DOE Human Genome Program Contractor-Grantee Workshop VI.

Wright, T.J., S. Abmayr, M.S. Park, D.O. Rieke, C. Naranjo, B. Welsh-Bretinger, K. Denison and M.R. Altherr. 1997. From genomic sequence to protein expression: a model for functional genomics. DOE Human Genome Program Contractor-Grantee Workshop VI.

Wright, T. J., C. Naranjo, E. Ortega, and M.R. Altherr. 1997. Comparative analysis of a novel gene mapping into the Wolf Hirschhorn syndrome critical region on chromosome 4p16.3. Annual Meeting: American Society of Human Genetics.

Collins, J., C. Naranjo, A. Ford, D. Ricke and M.R. Altherr. 1996. Localization of Trapped exons onto human chromosome 16. Annual Meeting: Southwestern and Rocky Mountain Division of the American Association for the Advancement of Science. *Note: J.C. received the "Outstanding Undergraduate Research Presentation Award" for this paper.*

Altherr, M.R., K. Denison, M. Clemens, O. Quarrel and T.J. Wright. 1996. Molecular Overlap in Wolf-Hirschhorn and Pitt-Rogers-Danks syndromes. Annual Meeting: American Society of Human Genetics.

Wright, T.J., K. Denison, V. Johnson, E. Zackai and M.R. Altherr. 1996. High resolution analysis of the Wolf-Hirschhorn syndrome region on chromosome 4p16.3. HGM-96 and 4th International Chromosome 4 Workshop.

Wright, T.J., K. Denison, D. Ricke, S. Abmayr, J. Collins, M. Somer, T. Yang-Feng and M.R. Altherr. 1996. A transcript map of the 260 kp Wolf-Hirschhorn syndrome critical region. Annual Meeting: American Society of Human Genetics.

Perez-Castro, A.V., J. Wilson and M.R. Altherr. 1995. Comparative genomic organization of the mouse and human fibroblast growth factor receptor 3 (FGFR3) genes. Annual Meeting: American Society of Human Genetics.

Wright, T.J., K. Denison and M.R. Altherr. 1995. Redefinition and molecular analysis of the critical region for the Wolf-Hirschhorn syndrome locus. Annual Meeting: American Society of Human Genetics.

Figure 1. (a) Physical map of the distal segment of the short arm of human chromosome 4, 4p16. The genetic loci utilized in this study and in previous studies are shown above the line, and the probes used in this study are shown below the line. The distance from locus D4S81 to the telomere is approximately 4.5 megabases. The genetic distance between loci MSX1 and D4S10 is 3 centiMorgan (cM, a unit of genetic distance) and the gap in the cloned contig between loci D4S168 and D4S96 is approximately 60 kilobases. The segment between the paired diagonal lines represents a contig of overlapping clones. The critical region defined by previous studies is shown by a solid box, and the new critical region defined by patients included in our studies is shown within solid box by an open box. The cross-hatched portion of the open box is the area that can be excluded when considering the 4p-patient. (b) Second-tier cosmids used in our FISH analysis. The probes used in the second round of the FISH analysis are shown. The results of the analysis of patient cell lines defining the WHSCR are shown. Lines indicate the position of the deletion, and arrows indicate that the deletion continues either telomeric (CM) or centromeric (LGL7447).

Figure 2. Transcription map of the Wolf-Hirschhorn Syndrome Critical Region (WHSCR). A cosmid contig of the WHSCR and region immediately flanking is shown. The solid line indicates the position of the new WHSCR. The position and direction of transcription, where known, of complementary DNAs are shown below the WHSCR. The broken line represents the region that can be excluded when considering the 4p- patient.