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TITLE: Interchromosomal Associations that Alter Nf1 Gene Expression can Modify Clinical Manifestations of Neurofibromatosis 1

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14. ABSTRACT We have described a new form of epistasis in which direct, long range, physical interactions between genes, or gene-gene interactions mediated by specialized DNA binding proteins such as CTCF, lead to modification of phenotypic read-out. Using the associated chromatin trap (ACT) and chromosome conformation capture (3C) assays which are designed to assess physical propinquity, we investigated long range interactions of the human NF1 gene that are mediated by CTCF in normal cultured cells. Using chromosome immunoprecipitation, we found multiple CTCF binding sites on NF1 in cultured cells. We explored long range chromatin associations with each of 7 CTCF binding sites and identified 14 distinct long range interactions. Among the genes that were physically associated with NF1 (which is on chromosome 17) was ARF4 (ADP-ribosylation factor 4, a member of the RAS superfamily involved in membrane traffic, signal transduction and organelle integrity on chromosome 3p14.3. The relative expression of ARF4 was increased several-fold in cells from patients with neurofibromatosis compared to normal cells, suggesting that the interchromosomal interactions of NF1 regulate gene expression on chromosome 3p14.3. It will be of interest to study the potential contribution of these associated genes to the pathophysiology and clinical manifestations of neurofibromatosis 1.					
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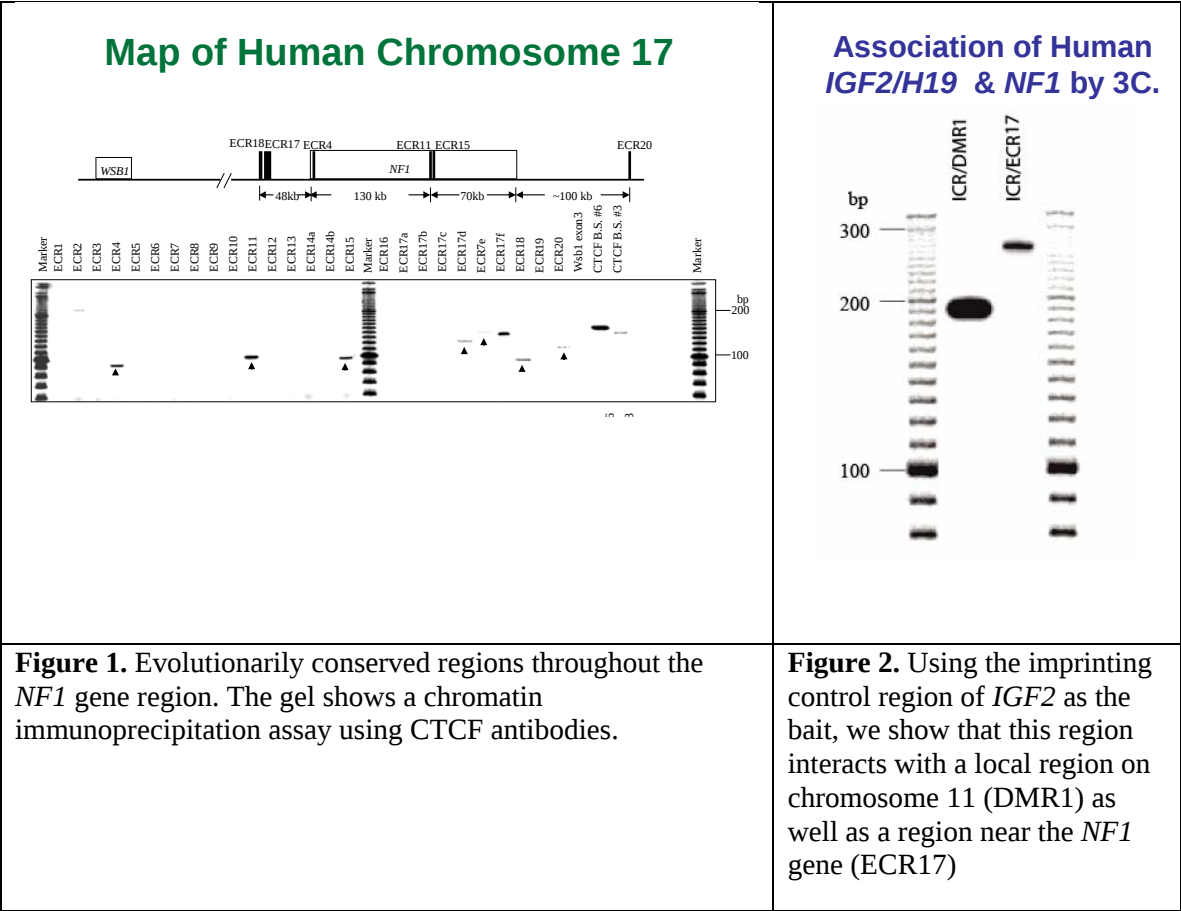
INTRODUCTION

One of the most remarkable aspects of neurofibromatosis 1 is the great variability in the expression of the disease, in which some affected patients may have few or mild manifestations, while others may have quite severe disease. Epistasis refers to a gene interaction in which gene A interferes with the phenotypic expression of gene B, in such a way that even if gene B is the “disease gene” (e.g., *NF1*), gene A may play an important or determining role in how the disease is manifest. We have described a new form of epistasis in which direct, long range, physical interactions between genes, or gene-gene interactions mediated by specialized DNA binding proteins such as CTCF, lead to modification of phenotypic read-out.(1)

BODY

Task 1: Characterize interactions between *NF1* and *IGF2* in normal human cells.

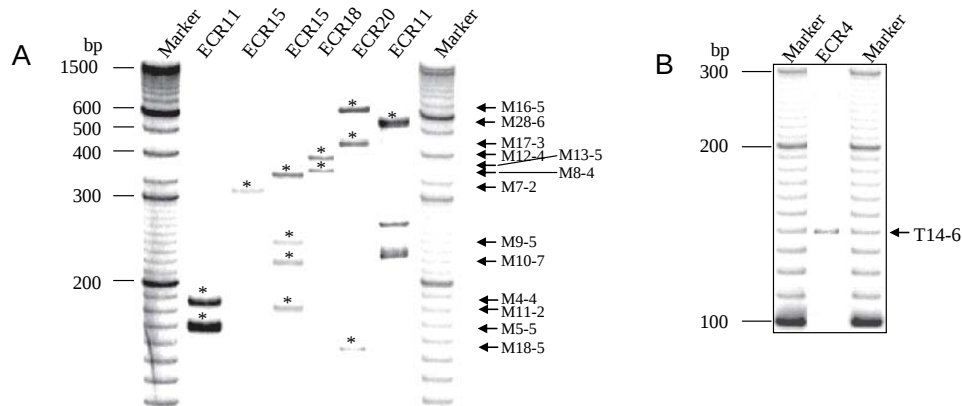
In our previous work, we had shown that the mouse *Nf1* gene interacted with *Igf2*. This interaction was mediated by CTCF.(2) We looked for evolutionarily conserved regions (ECR) between the mouse and human *NF1* genes, and discovered many throughout the gene region (Figure 1), many of which contain CTCF binding regions.(3) We explored the ability of ECR17, a CTCF binding region, to interact with other gene, and demonstrated that human *NF1* and *IGF2* physically interact in human cells, using the associated chromatin trap (ACT) and chromosome conformation capture (3C) assays (Figure 2).



Task 3: Search for new *NF1*-interacting partners

Using the ACT assay, we decided to begin our exploration of which other genes interacted with *NF1* in both normal cell lines and in cell lines derived from patients with neurofibromatosis. Using several CTCF-binding ECR regions, we have begun to elucidate many of these interacting genes, which are located on the multiple different chromosomes (Figures 3 and 4).

ACT Assay of *NF1* ECR Regions in Cultured Human Fibroblasts



Second PCR products of ACT assay at each ECR region of human *NF1* locus. Panel A shows the identified associated DNA fragment (*) with Msp I adaptor. Panel B shows the identified DNA fragment with Taq I adaptor.

Figure 3. *NF1* interacting genes are discovered by using different CTCF-binding ECRs. Distant gene segments are denoted by asterisks.

NF1 Interacting Genes

- T14-6, ECR4, DpnII-TaqI, chr17q11.2/chr3p14.3
 - SLMAP: sarcolemma associated protein
 - FLJ34969: hypothetical protein
 - ARF4: ADP-ribosylation factor 4
- M12-4, ECR18, DpnII-MspI, chr17q1.2/chr8q24.3
 - Putative nerve sheath tumor resistance 2 (Nstr2) locus on human chromosome 8q24.3 (Reilly KM et al., Cancer Res. 2006). ZNF250: zinc finger protein 250
- M13-5, ECR18, DpnII-MspI, chr17q11.2/chr1q21.3
 - ADAR: adenosine deaminase, RNA-specific isoform
 - CHRN2: cholinergic receptor, nicotinic, beta
- M11-2, ECR15 DpnII-MspI, chr17q11.2/chr14q32.13
 - TCL1A: T-cell leukemia/lymphoma protein 1A
- M28-6, ECR11, EcoRI-MspI, chr17q11.2/chr17q11.2
 - SUZ12P: suppressor of zeste 12 homolog pseudogene
- M8-4, ECR15 DpnII-MspI, chr17q11.2/chr22q11.23
 - Ral-GDS related protein Rgr, Contains 1 Ras-GEF domain

Figure 4. A sampling of the *NF1* interacting genes discovered by the ACT assay in Figure 3.

We became particularly interested in the interaction of *NF1* and *ARF4* (ADP-ribosylation factor 4, a member of the RAS superfamily involved in membrane traffic, signal transduction and organelle integrity). We confirmed the ACT data which suggested a physical interaction by directly demonstrating the interaction of one *NF1* allele with one *ARF4* allele using FISH analysis (Figure 5).

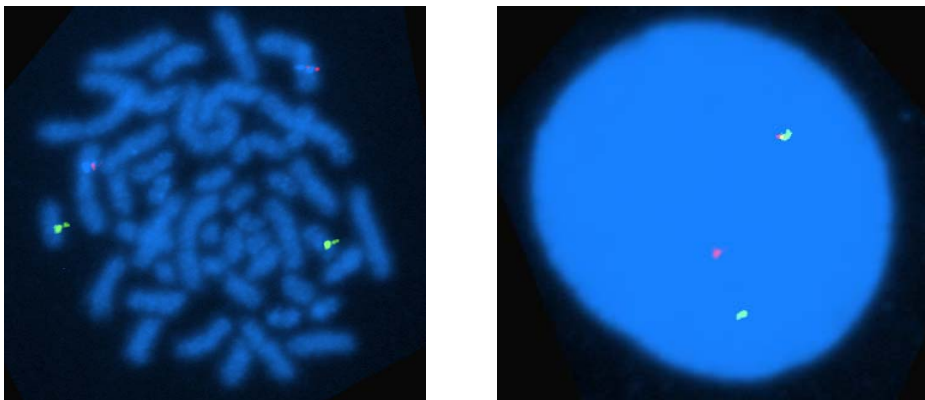


Figure 5. This FISH analysis shows *NF1* in green and *ARF4* in pink. On the left, two alleles of each gene are seen in mitotic chromosomes. In an interphase cell, one allele of each gene overlaps with the other, indicating a physical interaction between these two chromosomes.

We then began to examine the expression of *ARF4* in cell lines derived from patients with neurofibromatosis, reasoning that if the genes normally interact during interphase, this interaction might be abrogated to some extent in neurofibromatosis. As shown in Figure 6, our preliminary results suggest that *ARF4* gene expression is enhanced in neurofibromatosis, suggesting that *ARF4* may play a role in the manifestations of the disease.

Relative expression of *NF1* Associated Genes on Chr 3 in B cells from Neurofibromatosis Patients

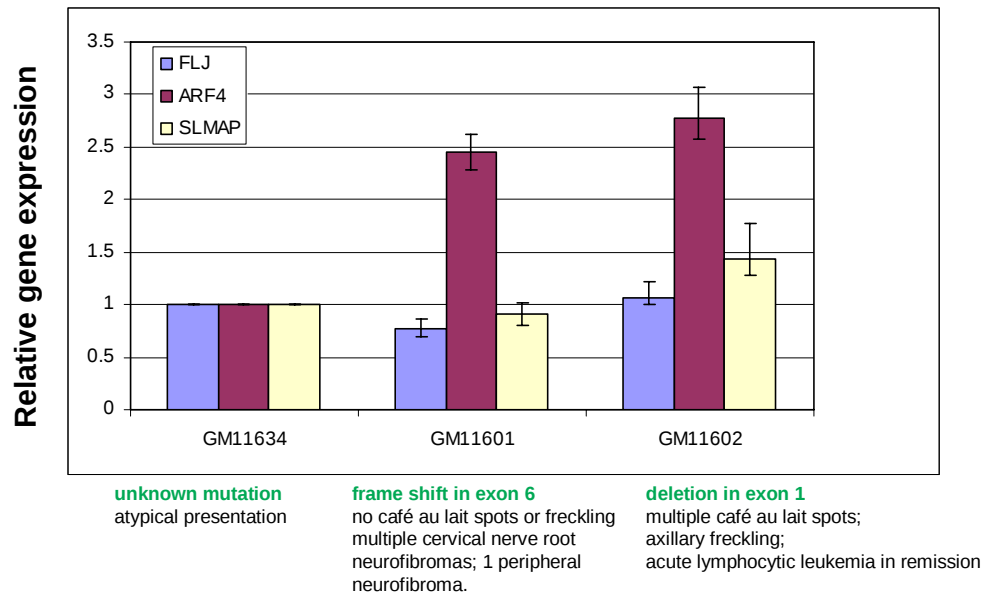


Figure 6. *ARF4* in neurofibromatosis.

KEY RESEARCH ACCOMPLISHMENTS

- The interactions between *NF1* and *IGF2* occur in both mouse and human cells.
- There are numerous CTCF binding sites in the *NF1* gene region.
- Using the associated chromosome trap assay, we identified a number of interchromosomal and inter-chromosomal interactions with the *NF1* gene, including an interaction with the *ARF4* gene on chromosome 3p14.3.
- FISH reveal over-lapping of one allele of *ARF4* with one allele on *NF1* in normal cells.
- *ARF* gene expression is increased in some cell lines derived from patients with neurofibromatosis 1.

REPORTABLE OUTCOMES

Hoffman AR and Ling JQ. Epigenetics and Long Range Interactions (Abstract S32). Presented at the 37th Annual Meeting of the Environmental Mutagenesis Society, Vancouver, BC, Sept 16-20, 2006.

Ling JQ, Hou A and Hoffman AR. *NF1* Interchromosomal Interactions in Normal and *NF1* Mutant Cell Lines (Abstract P21). Presented at The Children's Tumor Foundation Conference, Park City Utah, June 10-12, 2007.

CONCLUSIONS

1. *NF1* participates in numerous long range interchromosomal and interchromosomal interactions
2. When mutations in *NF1* occur, these interactions may be altered, leading to changes in gene expression.
3. The relevance of these gene interactions in regard to the clinical manifestations of neurofibromatosis 1 needs to be investigated.
4. The search for novel remote gene interactions with *NF1* promises to open up totally new ranges of therapeutic targets.

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3. Kim TH, Abdullaev ZK, Smith AD, et al. Analysis of the vertebrate insulator protein CTCF-binding sites in the human genome. *Cell* 2007;128(6):1231-45.

APPENDICES: none