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

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

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**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.

**Subject Terms:**
neurofibromatosis; epigenetics; epistasis; long range interactions
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).

Map of Human Chromosome 17

Figure 1. Evolutionarily conserved regions throughout the NF1 gene region. The gel shows a chromatin immunoprecipitation assay using CTCF antibodies.

Association of Human IGF2/H19 & NF1 by 3C.

Figure 2. Using the imprinting control region of IGF2 as the bait, we show that this region interacts with a local region on chromosome 11 (DMR1) as well as a region near the NF1 gene (ECR17)
**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
  - CHRN2B: 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


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.
REFERENCES


APPENDICES: none