Central Manchester University Hospitals NHS
NHS Foundation Trust

Characterising retrotransposon insertion mutations in Neurofibromatosis type 1 (NF1)

Naomi Bowers

Genomic Diagnostics Laboratory
St. Mary's Hospital, Manchester

- Multisystem autosomal dominantly inherited tumour predisposition syndrome
- Affects around 1/1,900-2,500 live births
- NCG funded service for atypical NF1
- Mutation detection is a challenge
  - The large size of the gene (58 coding exons)
  - The lack of mutational hotspots
  - The occurrence of a very diverse spectrum of mutation types
  - The presence of more than 30 unprocessed pseudogene sequences spread over the genome

- RNA-based mutation analysis
- Mutations are confirmed at the DNA level
- Sensitivity is 95% in NF1 patients meeting NIH criteria

EBioMedicine. 2016 May;7:212-20. doi: 10.1016/j.ebiom.2016.04.005. Epub 2016 Apr 13.

Comprehensive RNA Analysis of the NF1 Gene in Classically Affected NF1 Affected Individuals Meeting NIH Criteria has High Sensitivity and Mutation Negative Testing is Reassuring in Isolated Cases With Pigmentary Features Only.

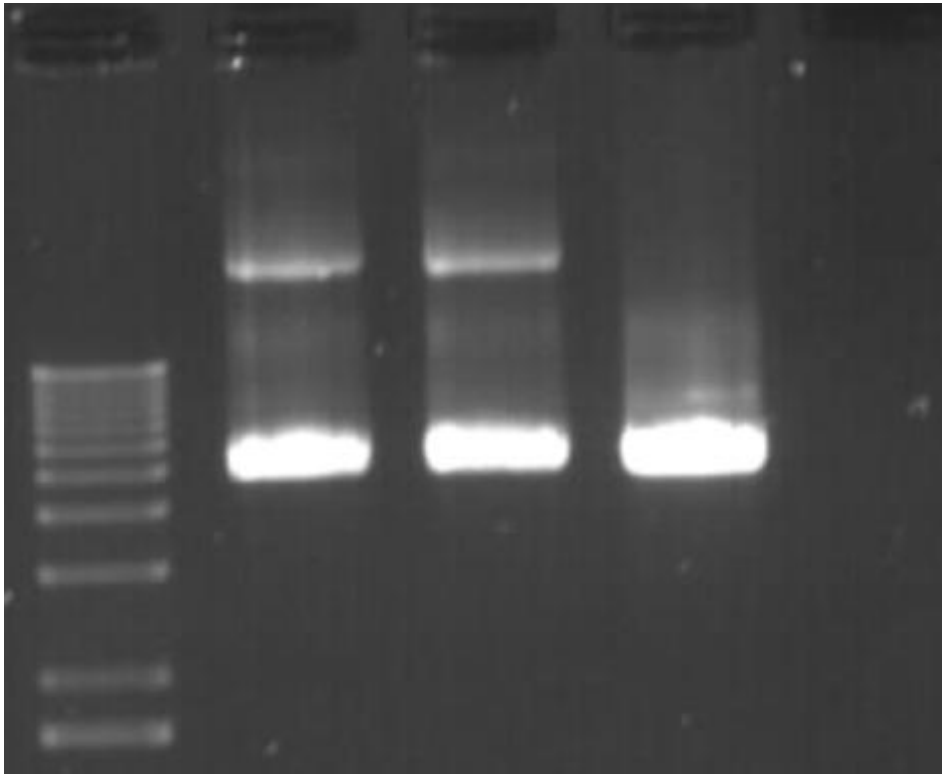
Evans DG[1], Bowers N[2], Burkitt-Wright E[2], Miles E[2], Garg S[2], Scott-Kitching V[2], Penman-Splitt M[3], Dobbie A[4], Howard E[2], Ealing J[2], Vassalo G[5], Wallace AJ[2], Newman W[6]; Northern UK NF1 Research Network, Huson SM[2].

- 2 cases
- 1 from our lymphocyte service
- 1 from our melanocyte service
  - Both fulfilled a clinical diagnosis of NF1
  - Both difficult to define at the DNA level

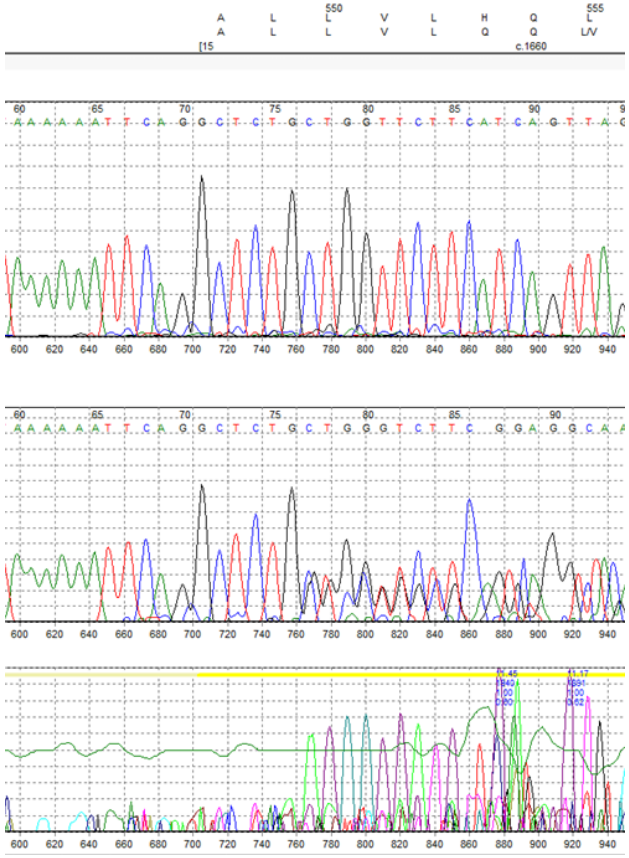
Cultured lymphocyte RNA Analysis

- Identified aberrant exclusion of exon 15 (r.1642_1721del)
- No causative mutation was identified in this patients genomic DNA sample
- Excluded procedural error
- Investigated the possibility of a structural rearrangement

- Long Range PCR of genomic region
- Abnormality found

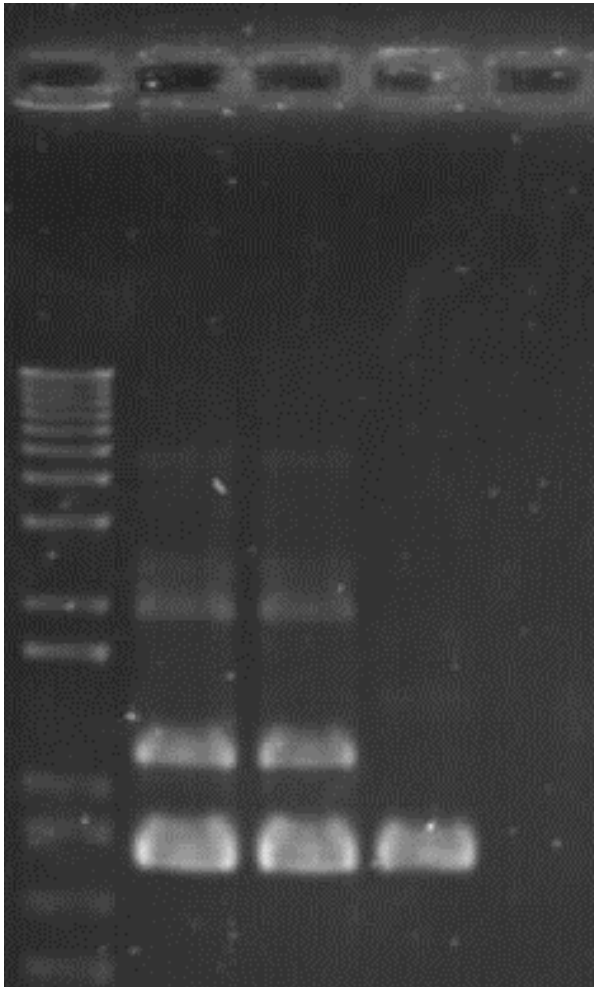


- End sequencing

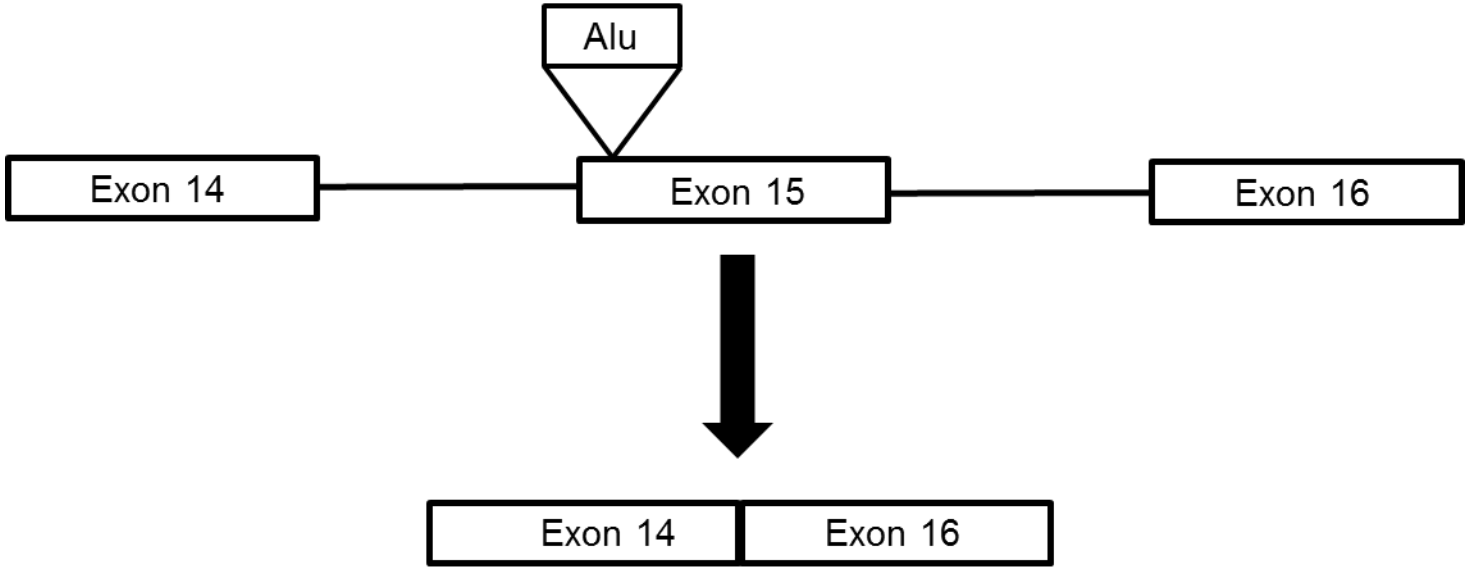


  - Identified novel sequence
  - Blast match to Alu repeat

- Long Range PCR to estimate the size



c.[1647_1648ins~366;1642-11_1647dup]

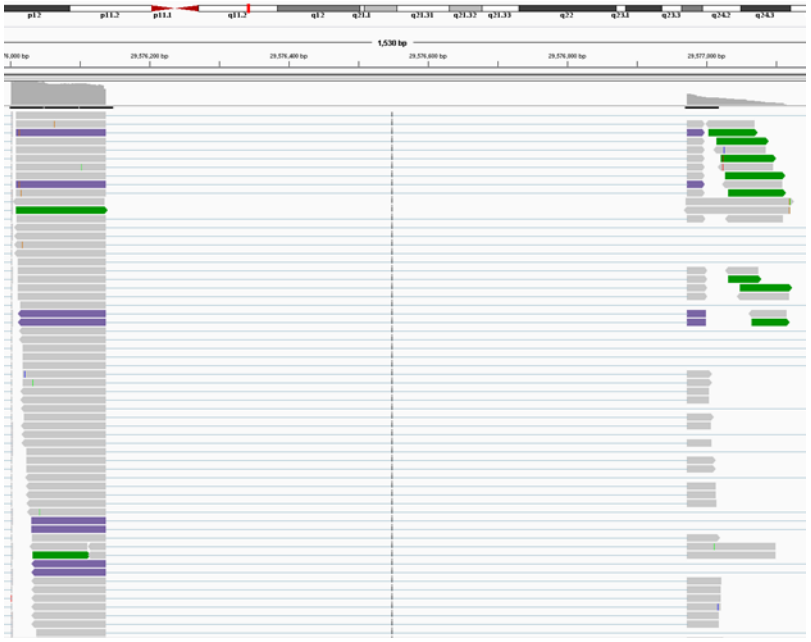


Mutation results in allele size differential preventing amplification using standard PCR

Cultured melanocyte RNA Analysis

- RNA analysis was complex
- Abnormality identified
  - Alternative transcript present at the same point
  - Analysis of triple sequence
- Intronic inclusion r.[4110_4111ins4110+836_?]
  - Unable to define the end point
  - Characterising at the DNA level failed
  - Unable to find any underlying abnormality when analysing the intronic region around the inclusion

Standard bioinformatics analysis showed the start of the intronic inclusion



Assembled Contig

GAGACGTTAACAGCATCATTGAAATCACCATTTTAAA
AGCTGTTTGAGGCTGCTCTATGTGAATTTTTTCACCA
TTGCTATTTTTAAAGATGTATCTGTTGAGGGAGGAGC
CAAGATGGCCGAATAGGAACAGCTCCGGTCTACAGCT
CCGGTCTACAGCTCCCAGCGTGAGCGACGCAGAAGAC
GGTGATTTCTGCATTTCCATCTGAGGCAACTTGCCAC
TCCCTACTGAATAAAGCTACAGTAAAAGAAAAAAAGG
AAAACAAAAAATCAGTGGTTAGCCAGCGTTTCCCTCA
GAACAGCATCGGTGCAGTAGGAAGTGCCATGTTCCTC
AGATTTATCAATCCTGCCATTGTCTCACCGTATGAAG
CAGGGATTTTAGATAAAAAGCCACCACCTAGAATCGA
AAGGGG

NF1 Intronic inclusion from intron 30

Unmapped

NF1 Exon 31

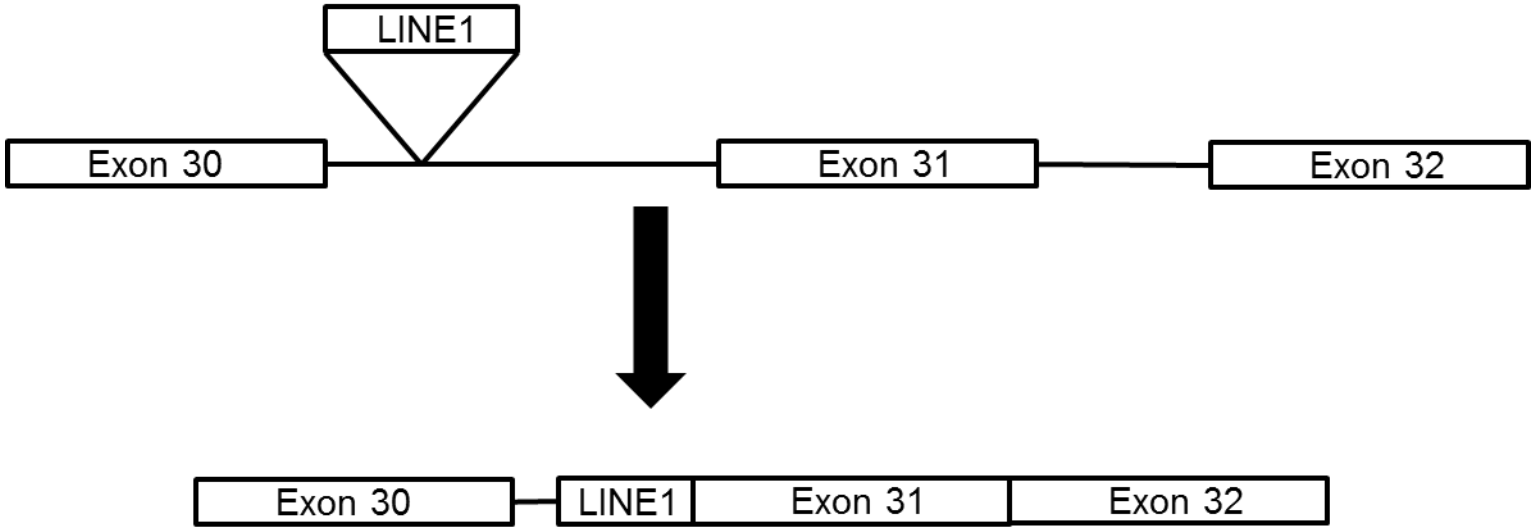
NF1 Exon 32

- Blast search of the unmapped contig showed 100% identity to LINE1 repeat element (long interspersed elements)
  - LINE1 element couldn't be mapped by the bioinformatic analysis because of sequence homology across the genome

- r.[4110_4111ins4110+836_4110+982;ins110]

- Long Range PCR of genomic region
  - Design was problematic due to sequence homology in other LINE1 repeats
    - Line1–insertion specific primer together with an intronic primer to specifically amplify and subsequently sequence the mutant allele
  - Confirmed size to be consistent with LINE1 repeat
  - End sequencing identified the insertion junctions

c.[4110+982_4110+983ins~6kb;4110+971_4110+982dup]



Insertion mutation is deep in the intron preventing identification using standard DNA screening

- Retrotransposon insertion mutations have previously been described in NF1

PLoS Genet. 2011 Nov;7(11):e1002371. doi: 10.1371/journal.pgen.1002371. Epub 2011 Nov 17.

The NF1 gene contains hotspots for L1 endonuclease-dependent de novo insertion.

Wimmer K[1], Callens T, Wernstedt A, Messiaen L.

- These 2 cases brought to our attention that our standard techniques would miss them when occurring in other genes
- These mutations are likely to comprise at least some of the undetectable mutations in other diseases