Accurate Transmission Risk Assessment in Potential Mosaic Neurofibromatosis Type 2 (NF2)

Jonathan Williams [1], Charlotte Noakes [1], Sirisha Hesketh [1], Sarah Steele [1], Penny Clouston [1], Carolyn Campbell [1], Dorothy Halliday [2]

[1] Oxford Regional Genetics Laboratories, Oxford University Hospitals NHS Trust

[2] Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust

Introduction

The majority of pathogenic *NF2* variants detected in sporadic Vestibular Schwannomas (VS) are undetectable in DNA extracted from lymphocytes. In contrast, an NF2 patient meeting clinical diagnostic criteria, with a unilateral VS diagnosed aged 20-29 and no *NF2* mutation identified in blood, has a 7% empiric offspring risk, due to somatic mosaicism [1,2]. Recent data using NGS, better able to identify lower levels of somatic mosaicism in blood, would suggest this figure to be an overestimate (Gareth Evans personal communication).

Here, we present the case of a 29 year old male presenting with unilateral VS. Initial analysis of tumour DNA using targeted NGS and MLPA (performed at the Manchester Centre for Genomic Medicine) detected two pathogenic single nucleotide variants (SNVs) in *NF2* in conjunction with a large deletion incorporating the entire gene. The reporting laboratory highlighted the likelihood of these findings representing a multi-focal tumour and the possibility of our patient being mosaic for NF2.

We have now used a combination of molecular techniques to refine this individual's transmission risk and elucidate the natural history of tumour development.

Array CGH Analysis

Array CGH of tumour DNA revealed the presence of two large mosaic deletions of 22q representing almost complete monosomy (Figure 1). Both deletions were undetectable in DNA extracted from blood or semen. We concluded that even if this individual were to be germline mosaic for this large imbalance, the live-born risk associated with it would be essentially nil.

Importantly, development of NF2 has been associated with constitutional rearrangements of chromosome 22 predisposing to loss of one copy of this chromosome; the risk of a low level mosaic rearrangement in our patient was further reduced by the analysis of 100 metaphase cells from cultured lymphocytes.

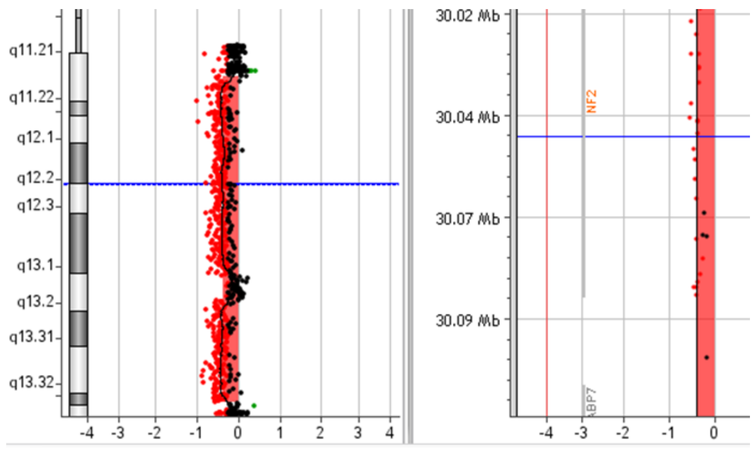

Figure 1

Array CGH analysis of tumour DNA highlighted the presence of two adjacent deletions of 22q, representing almost complete monosomy for this chromosome (including NF2)

Ultra-Deep NGS of Semen DNA

The pathogenic *NF2* SNVs were targeted for ultra-deep NGS in tumour and semen DNA using two overlapping ~7 kb long range PCR fragments followed by library preparation using Illumina NextEra XT and sequencing on the Illumina MiSeq platform. Analysis of tumour DNA confirmed the presence of c.363+1G>A at ~27% and c.432C>A at 13% consistent with the original report.

At a vertical read depth of >10,000 for each amplicon, c.363+1G>A was detectable in semen DNA at a maximum frequency of 0.09% and c.432C>A at 0.023% significantly reducing the empirical transmission risk for either variant in this individual.

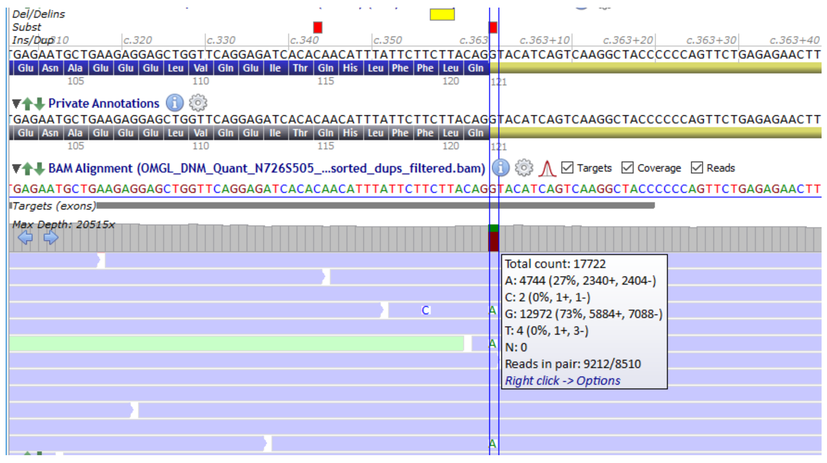

Figure 2

A portion of a BAM file alignment for the first long range PCR amplicon confirming the presence of c.363+1G>A at ~27% in tumour DNA

Variant Phasing

A comparison of variant frequency between tumour and semen DNA at a SNP (rs9614024) located between the *NF2* SNVs determined the large 22q deletion to involve the chromosome carrying the minor 'A' allele. We next sought to determine phase of each of the SNVs with respect to rs9614024 using the LALA-PCR technique [3].

As shown in Figure 3, enriching for each of the variants using an allele specific primer resulted in a corresponding drop of the A allele signal at the SNP position in Sanger sequencing traces. These results are consistent with each SNV arising on the same chromosome and opposite to the deletion event. We propose that the most likely sequence of events in tumour development is a first 'hit' in *NF2* consisting of the large 22q deletion event with the SNVs each acting as a second 'hit' in a separate tumour focus.

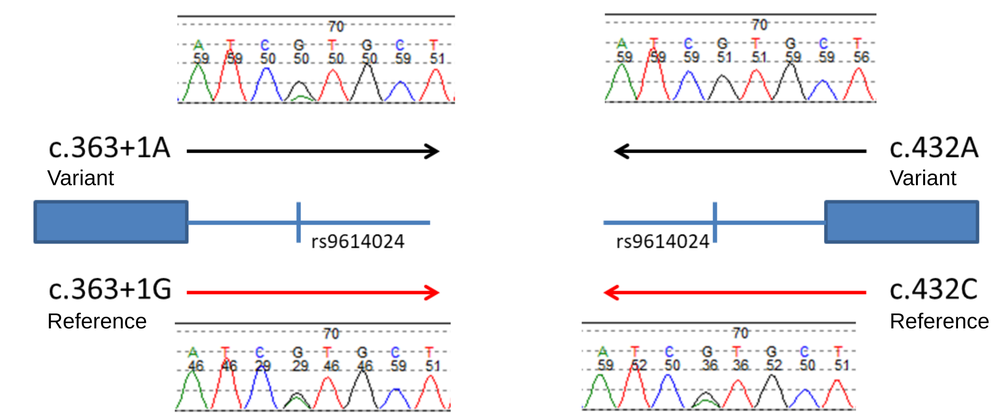

Figure 3

LALA-PCR analysis confirming the phase of each NF2 variant with respect to rs9614024

Conclusion

Deep sequencing of semen DNA has reduced this individual's empirical transmission risk of either *NF2* SNV to <0.1%. Evidence from phasing experiments suggests each SNV to be a tumour specific mutation further reducing the risk of this individual having offspring with NF2, particularly when considering the near zero live-born risk of the first hit 22q deletion.

References: [1] Evans *et al.,* 2007. *Journal of Medical Genetics*, **44**, pp.424-428; [2] Evans DG, *et al*, 2012. *Clinical Genetics*, **82**, pp.416–424. [3] Wu *et al.,* 2005. *Human Mutation*, **26**(4), pp.393-394