St George's University Hospitals NHS
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# A mosaic *KRAS* G12D variant in a tissue from a plexiform neurofibroma in a patient with *PTPN11* variant positive Noonan syndrome.

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## Abstract:

The Rasopathies are a group of highly variable developmental disorders caused by germline variants in the RAS-MAPK signalling pathway. They are characterised by distinctive dysmorphology, cardiac abnormalities, short stature and developmental delay. Roughly 50% are caused by variants in the *PTPN11* gene.

A 34 year old male with a clinical diagnosis of Noonan syndrome due to short stature, ptosis, undescended testes, webbed neck and low hair line, presented with a large plexiform neurofibroma causing discomfort.

Analysis of blood DNA on a 23 gene Rasopathy panel identified a c.923A>G p.(Asn308Ser) germline pathogenic variant in *PTPN11*, accounting for the patient's Noonan features but not the presence of the neurofibroma. A mosaic c.35G>A p.(Gly12Asp) *KRAS* variant was detected at an allele frequency of approximately 4% in tissue from the plexiform neurofibroma.

Plexiform neurofibroma are rarely seen in Noonan syndrome. They are common in neurofibromatosis type 1, another Rasopathy. We believe that the mosaic *KRAS* variant confers a 'second hit' in the formation of the plexiform neurofibroma.

## Case Presentation:

34 year old male presented with short stature, ptosis, easy bruising, undescended testes, webbed neck, low hair line, hypermobile joints along with a large plexiform neurofibroma causing him discomfort.

Neurofibromatosis-Noonan syndrome was suspected.

DNA was extracted from the patient's blood and sent for *NF1* testing and Rasopathy panel.

## The Rasopathies:

A group of congenital developmental syndromes caused by defects in the RAS-MAPK signalling pathway. Characterised by craniofacial dysmorphism along with cardiac, brain, skin, eye and musculoskeletal abnormalities.

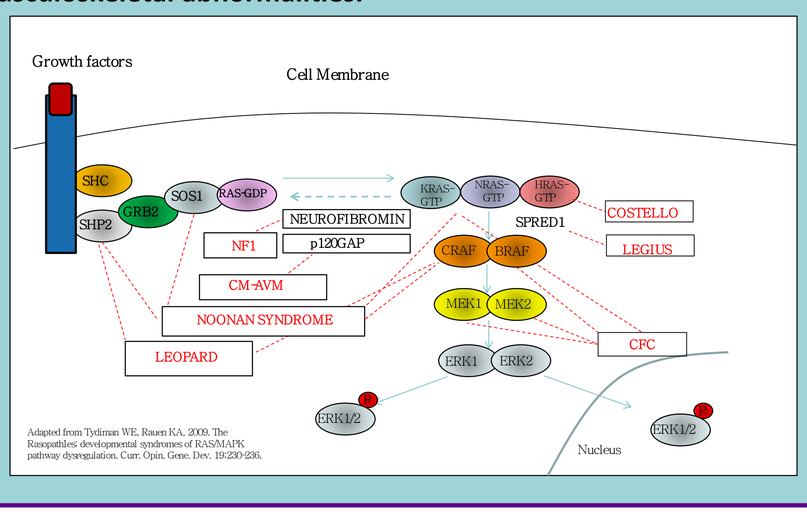



**Figure 1**. The RAS/MAPK pathway defects in the Rasopathies

## Genetic Testing:

No pathogenic variant detected in either *NF1* or *SPRED1* genes ruling out neurofibromatosis.

A c.923A>G p.(Asn308Ser) heterozygous pathogenic variant was detected in *PTPN11,* the gene encoding the SHP2 non-receptor protein tyrosine phosphatase. Up to 50% of cases of Noonan syndrome are caused by gain-of-function variants in *PTPN11*. These variants mainly disrupt the interaction of the N-SH2 and PTP domains, required to keep the protein in the inactive state.

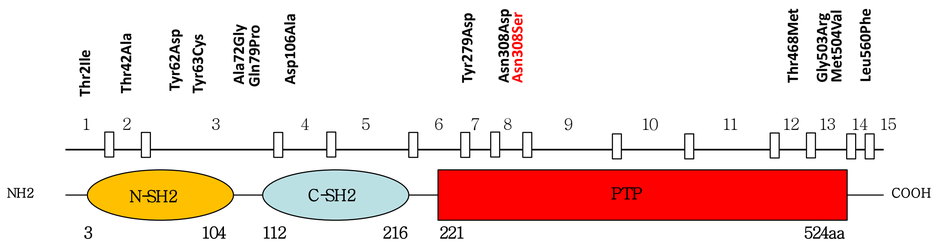



**Figure 2**. Schematic of the SHP2 protein and position of pathogenic, Noonan Syndrome causing variants

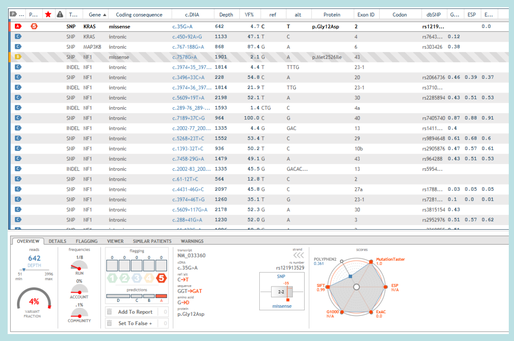
**This well characterised pathogenic variant accounts for the patient's classical Noonan features but NOT his plexiform neurofibroma.**

## Testing of Neurofibroma Tissue Sample

The plexiform neurofibroma was surgically removed from the patient's forehead and a sample sent for further genetic analysis on the Rasopathy panel at SGH.

A low level mosaic *KRAS* c.35G>A p.(Gly12Asp) pathogenic variant was detected at a 4% variant fraction. This variant is more commonly associated with human cancers such as lung, colorectal and pancreatic cancer than congenital syndromes.

A)



B)

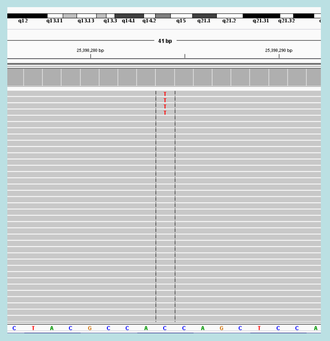
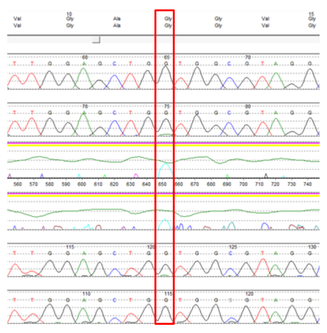



**Figure 3. A)** Sophia DDM analysis of NGS data from the patients neurofibroma DNA showing a low level *KRAS* G12D variant. **B)** Schematic representation of NGS reads covering the c.35 nucleotide position in our patient.

## Confirming the G12D variant:

The G12D variant is at too low a level to see clearly on Sanger sequencing. The Cobas *KRAS* QPCR-based assay was also used to confirm the variant.

A)



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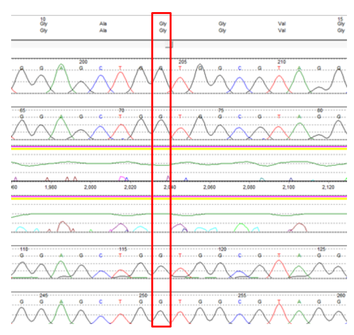

**Figure 4**. Sanger sequencing of *KRAS* exon 2 in DNA from the patient's neurofibroma **(A)** and from the patient's blood **(B)**. The c.35G>A can be seen at very low level only in the neurofibroma extracted DNA sample

## The Mosaic Rasopathies:

Mosaic G12D variants have previously been identified in an expanding group of congenital syndromes termed the "mosaic rasopathies". These include the epidermal nevi syndromes keratinocytic and sebaceous nevus. Mosaic Rasopathies caused by variants commonly associated with human cancers such as G12D, are at increased risk of tumour development. Detection of these variants therefore allows for closer monitoring of the patient for cancerous cellular changes.

## The Second Hit Hypothesis:

This case involves a patient with a clinical diagnosis of Noonan syndrome, later confirmed genetically by the presence of a pathogenic *PTPN11* gain-of-function variant. The patient unusually, also presented with a large plexiform neurofibroma. These benign nerve sheath tumours are commonly associated with neurofibromatosis but very rarely with Noonan syndrome.

Next generation sequencing of DNA extracted from a sample of the plexiform neurofibroma, identified a mosaic G12D pathogenic variant in the *KRAS* gene. We believe that on top of the *PTPN11* germline variant accounting for the patient's Noonan syndrome phenotype, this mosaic, post-zygotic *KRAS* variant confers a 'second hit' in the RAS-MAPK pathway, inducing the formation of the plexiform neurofibroma.

**References:**

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4) Wang H et al, 2015. KRAS G12d mosaic mutation in a Chinese linear nevus sebaceous syndrome infant. BMC Med Gen. 161;101.