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 dysmorphism, 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, low hair line, hypermobile joints along with a large plexiform neurofibroma. Genetic Testing:

DNA was extracted from the patient’s blood and sent for analysis of blood DNA on a 23 gene Rasopathy panel identified a c.923A>G p.(Asn308Ser) germline 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.

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.

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.

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.

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 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.

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

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

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

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.

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

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.

References:

2) Hafner C and Rauen KA, 2009. The Rasopathies. Adapted from WE, 2009. The Rasopathies - Developmental syndromes of RAS/MAPK pathway dysregulation. Cell Membrane PTP domains, required to keep the protein in the inactive state. 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.

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.

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 nevus syndrome keratinocytic and sebaceous nevus, Mosaic Rasopathies caused by variants commonly associated with human cancers such as lung, colorectal and pancreatic cancer than congenital syndromes.

Genes that are often mutated in sporadic cancers are rarely seen in Noonan syndrome. They are common in lung, colorectal and pancreatic cancer than congenital syndromes.

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