Identification of a Novel Noonan Syndrome with Cardiac Hypertrophy-Susceptibility Gene Using Whole Exome Sequencing (WES) and Trio-Based Genomic Subtraction

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Background
Noonan syndrome (NS) is an autosomal dominant, genetically heterogeneous disorder resulting in a characteristic clinical phenotype of facial dysmorphisms, short stature, and congenital cardiac defects. Concomitant cardiac ventricular hypertrophy (CVH) with ventricular outflow tract obstruction (VOTO) is seen in approximately 20% of patients. Besides the most common NS-susceptibility gene, PTPN11, several other NS-susceptibility genes (i.e. RAF1 and BRAF) that encode proteins involved in the RAS-mitogen-activated protein kinase (MAPK) signaling pathway have been identified in patients with NS. Specific gain-of-function mutations in these genes result in NS with CVH/VOTO due to activation of ERK signaling.

Methods
Whole exome sequencing (WES) and trio-based genomic filtering was performed on a 15-year-old female diagnosed with CVH and VOTO in infancy and subsequently NS at 8 years of age. Previously, commercially available gene test panels for both hypertrophic cardiomyopathy (HCM) and NS did not identify any variants in known HCM- or NS-associated disease genes. WES was performed using Illumina Hi-seq and analyzed using bio-informatics TREAT protocol on the patient and her asymptomatic parents with normal echocardiograms. Sequence data was analyzed using Ingenuity Variant Analysis Software. Filters were designed to identify de novo variants (absent in both parents), absent in publically available exomes (NHLBI, 1000 genomes), and associated with known NS/HCM-associated signaling pathways.

Results
After bioinformatic analysis, exclusion of common variants, and trio-based familial subtraction, WES identified 103 sporadic de novo, candidate variants in 81 genes. Of these, 16 variants resided within 13 genes that encode proteins involved in known NS/HCM pathways. However, only 1 missense variant (G23V) in MRAS-encoded RAS-related protein M-ras (MRAS) - involved a gene that directly interacts with the NS-associated RAF1 and BRAF proteins of the RAS/MAPK pathway. The other 12 genes encoded proteins that only interacted indirectly with these pathways. The G23V-MRAS variant resides in the critical GTP-binding motif of the protein and was predicted by in silico tools to be damaging and result in a gain-of-function.

Conclusion
Herein, we provide preliminary evidence for a novel NS-susceptibility gene, MRAS, discovered by WES and subtraction of parental exomes. MRAS plays a crucial role in pro-hypertrophic RAS/MAPK pathways and interacts with known NS-associated proteins, RAF1 and BRAF. Further studies are necessary to determine whether this sporadic mutation, G23V-MRAS, is disruptive functionally in a gain-of-function manner and to determine the contribution of MRAS mutations among patients currently classified with genotype-negative NS.