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Prenatally diagnosed *RASA1* deletion: an incidental finding that uncovers a family history

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1. Introduction

- Red birthmarks (hemangiomas) occur in ~40% of newborns and appear when an overgrowth of capillaries is present near the surface of the skin. While the majority resolve without intervention, some can persist and may require additional medical care.
- A subset of patients may have an underlying genetic condition: autosomal dominant capillary malformation-arteriovenous malformation syndrome (CM-AVM). The *RASA1* gene is responsible for 50% of cases with CM-AVM, the majority of which are mutations resulting in haploinsufficiency.
- The phenotype is characterized by the presence of small capillary malformations on the face and limbs with or without AVMs, arteriovenous fistulas (AFVs), and rarely Parkes Weber syndrome. Other findings may include lymphatic malformations and cardiac overload/failure.
- In severe cases, medical management may include a multidisciplinary team including dermatology, cardiology, radiology, surgery and/or orthopedics. Penetrance of the condition is 90-99%. Familial inheritance is noted in 70% of cases, however larger deletions encompassing *RASA1* are usually *de novo* and have additional phenotypic effects from involvement of other pathogenic genes, namely *MEF2C*.

2. Methods

- SNP microarray methodology: SNP microarray analysis was performed using the Affymetrix (now Applied Biosystems® Microarray Analysis) Cytoscan® HD platform which uses over 743,000 SNP probes and 1,953,000 NPCN probes with a median spacing of 0.88 kb. 250ng of total genomic DNA was digested with NspI and then ligated to NspI adaptors, respectively, and amplified using Titanium® Taq with a GeneAmp® PCR System 9700. PCR products were purified using AMPure® beads and quantified using NanoDrop® 8000. Purified DNA was fragmented and biotin labeled and hybridized to the Affymetrix (now Applied Biosystems® Microarray Analysis) Cytoscan® HD GeneChip®. Data was analyzed using Chromosome Analysis Suite. The analysis is based on the GRCh37/hg19 assembly.
- qPCR methodology: qPCR analysis was performed using the QuantStudio® 7 Flex Real-Time PCR system (Applied Biosystems®) in conjunction with the VeriQuest® Fast SYBR Green qPCR Master Mix (2X) (Thermo Scientific®). All samples were run in triplicate. To determine copy number, primers specific to an amplicon within a CNV identified by microarray analysis were compared to amplicons specific to two housekeeping genes (*RNase1* nd *TBP*). Two normal controls, one female and one male, were run for all cases with validated primers. For cases with unvalidated primers, a positive control was included. Genomic copy number determinations for specific CNVs were made as follows: qPCR value of 0=0 copies, from .06 to 1.4=1 copy, 1.6 to 2.4=2 copies, 2.6 to 3.4=3 copies and 3.6 to 4.4=4 copies.

3. Case Report

We present the case of a proband whose mother was a 23 year-old primigravida who presented for genetic counseling at 11 weeks 6 days' gestation secondary to absent nasal bone and increased nuchal translucency (3.8mm) on ultrasound. The mother reported a family history of distant relatives with intellectual and physical disabilities due to an unbalanced translocation, however no records could be obtained. The mother also had a notable personal history of Long QT syndrome (molecular testing confirmed). The mother opted for chorionic villus sampling (CVS). Aneuploidy FISH and SNP microarray were ordered on CVS to rule out any significant copy number or copy neutral imbalances in the fetus. Fetal diagnosis for Long QT was declined.

Figures

Figure 1: SNP microarray analysis was performed using the Affymetrix (now Applied Biosystems® Microarray Analysis) Cytoscan® HD platform and detected a detected a 3.02 MB interstitial microdeletion of 5q14.3 (83,884,771-86,902,728)x1 that included 3 OMIM genes (*COX7C*, *RASA1*, *CCNH*).

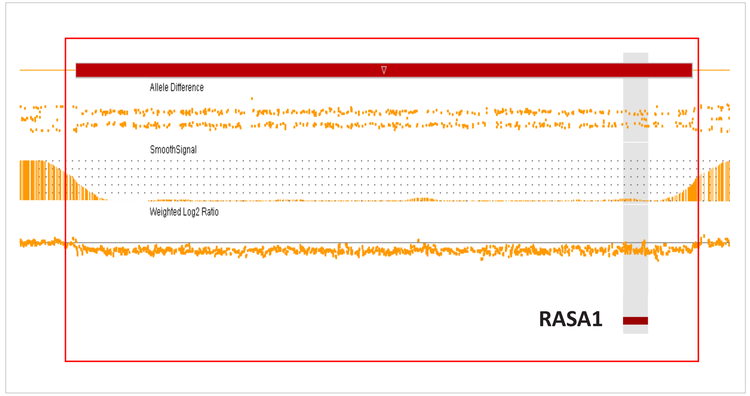

Figure 2: qPCR analysis was performed using the QuantStudio® 7 Flex Real-Time PCR system (Applied Biosystems®) in conjunction with the VeriQuest® Fast SYBR Green qPCR Master Mix (2X) (Thermo Scientific®). All samples were run in triplicate. To determine copy number, primers specific to an amplicon within a CNV identified by microarray analysis were compared to amplicons specific to two housekeeping genes (*RNase1* and *TBP*). Two normal controls, one female and one male, were run for all cases with validated primers. Follow up qPCR analysis targeting the deleted region in the proband analysis confirmed the deletion in the proband (**figure 2a**). Paternal analysis (**figure 2b**) also detected the deletion. Maternal analysis (**figure 2c**) was normal. FWD and REV qPCR primers were localized to the *RASA1* gene.

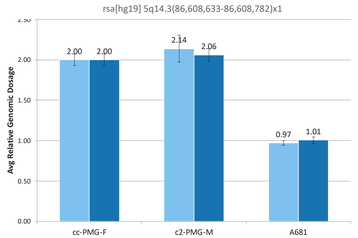

Figure 2b: qPCR analysis on the paternal specimen compared to two controls

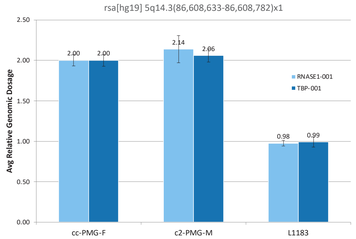

Figure 2a. qPCR analysis on the proband specimen compared to two controls

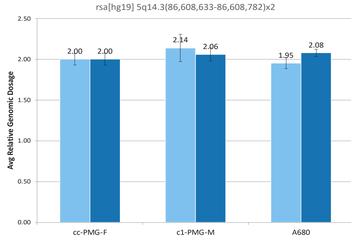

Figure 2c: qPCR analysis on the maternal specimen compared to two controls

4. Results

- Aneuploidy FISH reported normal male.
- SNP microarray run on cultured villi revealed an interstitial deletion of chromosome 5, at band 5q14.3 that involved 3 genes, including whole gene deletion of *RASA1* but not *MEF2C*.
- Results of parental qPCR testing revealed the father of the pregnancy carried the same 5q deletion involving *RASA1*. Maternal analysis was normal.
- No evidence seen on SNP microarray of imbalance related to verbal family history of translocation.
- Baby delivered without issue, minor hemangiomas but no outstanding features
- Postnatal referral to pediatric genetics recommended after delivery, and formal evaluation of child is pending, as well as paternal family follow-up

5. Discussion

- Parental follow-up discussion uncovered a seemingly benign family history reported by the father of the pregnancy, who described a large hemangioma on his tongue in childhood that interfered with the ability to close his mouth, along with other orthodontic complications including tongue-tie, wide spaced teeth, and under-bite.
- The father's mother, maternal aunt, and niece also had red raised birthmarks. Additionally, the niece had wide spaced teeth, tongue-tie, and an abnormal upper lip frenulum.
- As previously described by Wooderchak-Donahue et. al (see references), prior studies of *RASA1* have thus far only detected mutations and small exonic deletions within and on the promoter end of *RASA1*. Full gene deletions have only been seen to exist as part of a large contiguous gene deletion involving *MEF2C*, which presents with intellectual disability, behavioral abnormalities, and stereotypic movements akin to Rett syndrome. As such, all prior contiguous gene deletions have been *de novo*.
- This appears to be the first described case of an inherited *RASA1* whole gene deletion without contiguous deletion involving *MEF2C*. Therefore, whole gene deletions may be anticipated to have the same high inheritance percentage as mutations of *RASA1* when *MEF2C* is not included.

References

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3. Burrows, Patricia E et al. "Lymphatic abnormalities are associated with RASA1 gene mutations in mouse and man." Proceedings of the National Academy of Sciences of the United States of America vol. 110,21 (2013): 8621-6. PMID: 23650393.

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