186

The Wolf-Hirschhorn Critical Region… Not So Critical?

J. Schleede[1], M. Ciarlariello[2], V. Jaswaney[1], E. Ashkinadze[2], T. Rosen[2], P. Papenhausen[1]
1. Laboratory Corporation of America® Holdings/Integrated Genetics, 2. Rutgers-Robert Wood Johnson Medical School, Department of OB-GYN

Integrated GENETICS
LabCorp Specialty Testing Group

I. Introduction

Wolf-Hirschhorn syndrome is a well-described contiguous gene deletion syndrome that causes severe global developmental delays, seizures, growth retardation, microcephaly, and distinct craniofacial features described as a "Greek warrior helmet" appearance. Early chromosome studies identified a number of terminal 4p deletions with heterogeneous breakpoints in individuals diagnosed with Wolf-Hirschhorn syndrome. Initial genotype-phenotype correlation studies utilizing both fluorescence in situ hybridization (FISH) and microarray analyses refined the terminal 4p deletions to a minimal ~1.9 Mb critical region. However, questions still remained as to what genes were responsible for the individual phenotypes associated with Wolf-Hirschhorn syndrome.

To further refine the critical region, additional studies compared smaller overlapping deletions within the 1.9 Mb terminal 4p deletion. This analysis resulted in the characterization of the Wolf-Hirschhorn critical region (WHSCR). However, variable expressivity of phenotypes associated with deletions that included the WHSCR prompted researchers to re-examine the region ultimately leading to refinement to the WHSCR-2. The WHSCR-2 encompasses two genes: *NSD2* and *LETM1*. *NSD2* was initially thought to contribute to the abnormal facial features as well as the global developmental delay, while *LETM1* has been implicated in seizure development. However, recent studies have argued that deletions involving *NSD2* and *LETM1* may only contribute to a subset of the features of Wolf-Hirschhorn syndrome including growth deficiency, feeding difficulties, and speech and motor delays, suggesting that deletion of additional genes is required for full expression of the phenotype.

Here we present a case that further highlights the difficulty in establishing a minimal critical region for expression of phenotypes related to Wolf-Hirschhorn syndrome.

II. Materials and Methods

We received amniotic fluid and a maternal peripheral blood sample from a 39 year old female referred at 20 weeks gestation for prenatal microarray analysis. Paternal peripheral blood and buccal cells as well as peripheral blood from the paternal grandparents were also received for follow-up analyses.

DNA purification was performed using a Maxwell® 16 MDx Research Instrument (Promega®). Blood, and amniotic fluid, and samples were purified using a Maxwell® 16 Blood DNA Purification Kit. Buccal samples were purified using a Maxwell® 16 Buccal Swab LEV DNA Purification Kit.

Microarray analysis was performed using the Affymetrix® CytoScan® HD array [Affymetrix® and CytoScan® are Registered Trademarks of Affymetrix, Inc.]. This array contains approximately 2.695 million markers across the entire human genome. There are approximately 743,000 SNPs and 1,953,000 structural non-polymorphic probes (NPCNs).

qPCR analysis was performed using the QuantStudio™ 7 Flex Real-Time PCR system (Applied Biosystems®) in conjunction with the VeriQuest™ SYBR© Green qPCR Master Mix (Affymetrix®) with ROX (2X) (2000 rxn). All samples were run in triplicate using primers (FWD: GTGAAACAGGTAGTGAGTTGATCG, REV: AGCCTAGACCTCAGTCCATTTC) specific to an amplicon within the *FGFR3* locus, rsa[hg19] 4p16.3(1,797,193-1,797,302), in conjunction with three normal controls (one female and one male from Promega®, and one male from Affymetrix®) and one no template control. A primer pair specific to RNaseP was used as an internal control. Genomic copy number determinations for specific CNVs are 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.

III. Results

Proband: Microarray analysis was performed on DNA isolated from cultured amniocytes from a 39 year old female referred for testing at 20 weeks gestation due to multiple ultrasound abnormalities including renal hypoplasia and growth deficiency. Microarray analysis revealed a 748 kb interstitial deletion of 4p16.3 that includes 13 OMIM genes and spans the entire WHSCR-2 region (**Figure 1**). The deletion was confirmed by qPCR analysis using a deletion specific amplicon targeting the *FGFR3* locus (**Figure 2A**).

Maternal: Follow-up qPCR analysis of peripheral blood from the 39 year old female showed two copies of the *FGFR3* localized amplicon, ruling out a maternal carrier of the deletion (**Figure 2B**).

Paternal: Clinical evaluation at 40 years old revealed no apparent congenital anomalies and an above average intelligence. Contrary to what was expected, the paternal peripheral blood sample was positive for deletion of the *FGFR3* locus by qPCR analysis (**Figure 2C**). Deletion of the entire 748 kb region was confirmed by microarray analysis of the same peripheral blood sample (**Figure 3**). The deletion appeared to be present in 100% of cells in both the follow-up qPCR and microarray analyses. To test for possible tissue mosaicism, DNA was isolated from paternal buccal cells and assayed by microarray (**Figure 4**). The deletion appears to be present in 100% of buccal cells reducing the likelihood of tissue specific mosaicism.

Paternal grandparents: To determine the inheritance of the 748 kb deletion in the paternal carrier, qPCR analysis was performed on the paternal grandparents. Both grandparents showed two copies of the *FGFR3* localized amplicon suggesting the deletion occurred *de novo* in the paternal carrier (**Figure 5**).

Figure 1: Microarray analysis of DNA from amniotic fluid culture revealed a 748 kb interstitial deletion of 4p16.3 spanning 13 OMIM genes including the entire WHSCR-2 region.

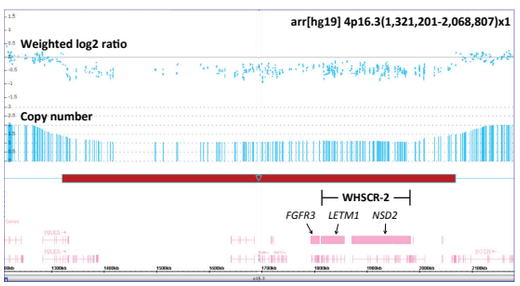


Figure 2: Confirmation and parental follow-up of the 748 kb 4p16.3 deletion by qPCR analysis using an amplicon specific to the *FGFR3* locus. (**A**) Deletion is confirmed in proband. (**B**) Maternal peripheral blood analysis is negative for deletion. (**C**) Paternal peripheral blood analysis is positive for deletion.

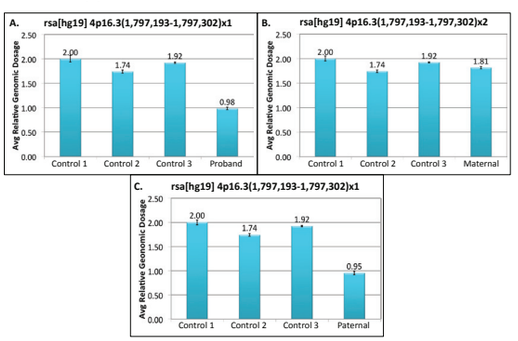


Figure 3: Paternal microarray analysis of peripheral blood confirms deletion of the entire 748 kb 4p16.3 interval observed in the proband.

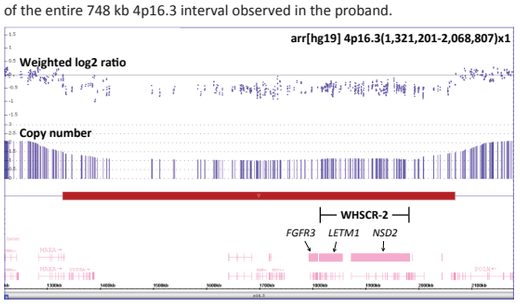


Figure 4: Paternal microarray analysis of buccal cells is positive for the 748 kb 4p16.3 deletion further confirming the paternal origin and reducing the likelihood of tissue specific mosaicism.

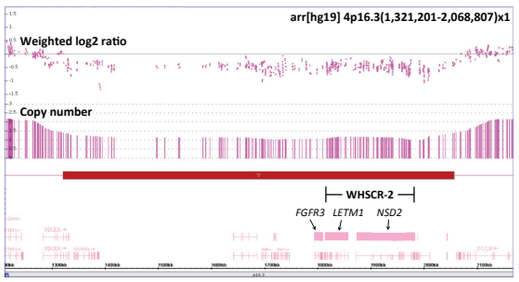
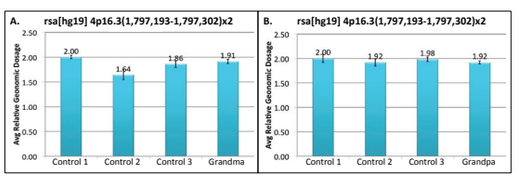


Figure 5: Follow-up of the 748 kb 4p16.3 deletion in the paternal grandparents by qPCR analysis. Peripheral blood analysis from both the paternal grandmother (**A**) and grandfather (**B**) showed two copies of the *FGFR3* specific amplicon consistent with the deletion occurring *de novo* in the paternal carrier.



IV. Conclusions

The initial 748 kb deletion of 4p16.3 found by microarray analysis of cultured amniocytes, combined with the reported ultrasound abnormalities, is highly suggestive of a prenatal diagnosis of Wolf-Hirschhorn syndrome. However, the deletion was found to be inherited from a clinically normal paternal carrier. These results indicate that although the deletion may have contributed to the phenotypic abnormalities in the fetus, it was not sufficient in the paternal carrier to lead to the expression of phenotypes associated with Wolf-Hirschhorn syndrome or the subset of features found in individuals with smaller WHSCR-2 deletions.

Previously described individuals with smaller overlapping 4p16.3 deletions that include the WHSCR-2 region present with abnormal phenotypic characteristics including growth deficiency, feeding difficulties, and motor and speech delays. However, these individuals lack seizures and the typical craniofacial findings associated with Wolf-Hirschhorn syndrome. All previously characterized overlapping deletions that include the WHSCR-2, in cases where parental analysis was available, have been found to be *de novo*. The paternal carrier presented here is the first unaffected individual reported to carry a deletion that spans the WHSCR-2 critical region. One explanation as to why the paternal carrier is unaffected is that deletion of the WHSCR-2 region is associated with a greater degree of variable expressivity than what has been reported previously, further implicating the involvement of additional genes in phenotypic manifestation. A second explanation is that given the deletion occurred *de novo*, the paternal carrier is a tissue specific mosaic. Although, no mosaicism was observed in either the peripheral blood or buccal analysis, the possibility that other tissues do not carry the deletion cannot be entirely ruled out.

These results underline the complexity of high resolution prenatal genetic diagnoses. Although previous analyses from the literature can be useful in interpreting the phenotypic consequences of genetic variation, parental follow-up remains one of the most useful tools for variant interpretation. These results also demonstrate the importance of genetic counseling to ensure patients receive accurate information pertaining to the relationships between penetrance, variable expressivity, mosaicism and phenotypes associated with genetics abnormalities.

V. References

Maas et al., *J Med Genet*. 2008 Feb; 45(2):71-80. PMID: 17873117
Zollino et al., *Am J Hum Genet*. 2003 Mar; 72(3):590-7. PMID: 12563561
Rodriguez et al., *Am J Med Genet A*. 2005 Jul 15; 136(2):175-8. PMID: 15948183
Hammond et al., *Eur J Hum Genet*. 2012 Jan; 20(1):33-40. PMID: 21792232
Van Buggenhout et al., *J Med Genet*. 2004 Sep; 41(9):691-8. PMID: 15342700
Wright et al., *Hum Mol Genet*. 1997 Feb; 6(2):317-24.PMID: 9063753
Endele et al., *Genomics*. 1999 Sep 1; 60(2):218-25. PMID: 10486213
Andersen et al., *Eur J Hum Genet*. 2014 Apr; 22(4):464-70. PMID: 239633003.

LabCorp