MICROARRAY REPORT:
Deletion/Duplication Analysis of the KRIT1 Gene via aCGH

SUMMARY:  POSITIVE: Heterozygous for a large deletion in KRIT1

RESULTS AND INTERPRETATIONS: This patient is heterozygous for a deletion of ≥ 62 kb length within the KRIT1 (NM_194456.1) gene. This deletion encompasses exons 1 to 18 and has breakpoints proximal to the KRIT1 gene and within intron 18 corresponding to a minimum deletion boundary via aCGH of chr7:91,835,157-91,897,620 (GRCh37/hg19).

To our knowledge, this deletion has not been reported in the literature, but is the type expected to be pathogenic (see for example Riant et al. Neurogenetics 14:133-141, 2013).

This result should be interpreted in the context of clinical findings, family history and other laboratory reports. All genetic tests have limitations. Please see limitations and other notes for this particular test on pages 2 - 3.

ISCN Nomenclature: arr[hg19] 7q21.2(91,835,157-91,897,620)x1

NOTES: CCMs exhibit autosomal dominant inheritance with high, although incomplete, lifetime penetrance. Testing for the KRIT1 deletion in relatives of this patient should therefore be carefully considered.

METHODS: Genomic DNA from this individual and gender-matched reference sample were labeled and hybridized on a microarray containing probes across the entire KRIT1 gene.
Limitations of Test and Other Test Notes
Interpretation of the test results is limited by the information that is currently available. Better interpretation should be possible in the future as more data and knowledge about human genetics and this specific disorder are accumulated.

Only the indicated gene or genes were analyzed. Test reports contain no information about other regions of the genome, including genes that are not requested, genes that have pseudogene copies and genes that are not targeted on the PreventionGenetics’ HDGC custom designed aCGH. This test does not provide any information about deletions or duplications within repetitive elements.

Any copy number change smaller than 300bp within a targeted large exon or 2kb in the intronic regions may not be detected by our array.

Balanced translocations or inversions within a targeted gene, or large unbalanced translocations or inversions that extend beyond the genomic location of a targeted gene are not detected.

In nearly all cases, our ability to determine the exact copy number change within a targeted gene is limited. In particular, when we find duplication within a targeted gene, we cannot be certain that the region is duplicated, triplicated etc. In duplication cases, we are unable to determine the genomic location or the orientation of the duplicated segment with respect to the gene. In particular, we cannot determine if the duplicated segment is inserted in tandem within the gene or inserted elsewhere in the genome. Similarly, we cannot determine the orientation of the duplicated segment (direct or inverted), and whether it will disrupt the open reading frame of the given gene.

Our ability to detect minor copy number change, due for example to somatic mosaicism is limited. Copy number changes that are present in less than about 30-40% of the patient’s cells may not be detected.

Unless otherwise indicated, the PreventionGenetics’ custom designed aCGH results are based on DNA isolated from a specific tissue (usually leukocytes). Test reports contain no information about copy number changes in other tissues.

We cannot be certain that the reference sequence(s) are correct. Exons, for example, may be misidentified. In cases where the genomic and mRNA sequences disagree, we used the genomic sequence to design the probes.

We have confidence in our ability to track a specimen once it has been received by PreventionGenetics. However, we take no responsibility for any specimen labeling errors that occur before the sample arrivers at PreventionGenetics.

Normal findings within a targeted gene do not rule out the clinical diagnosis of a genetic disease.

Genetic counseling to help explain test results to the patients and to discuss reproductive or medical options is recommended.

Methodology
As required, genomic DNA (gDNA) is extracted from the patient specimen. Equal amounts of gDNA extracted from blood samples from patient (Test) and gender matched control (Reference) samples are differentially labeled with fluorescent dyes. A unique set of oligo is added to each ‘Test’ sample as spike-in. This is a quality control measure that enables us to prevent any sample mix up during the processing of the samples in the microarray lab. Following labeling both the ‘Test’ and the ‘Reference’ samples are purified, quantified and combined in equal amounts before hybridization on the microarray slides. After 22hrs of hybridization at 65°C, the slides are washed and scanned immediately using manufacturer’s protocol.

PreventionGenetics’ high density gene-centric (HDGC) aCGH contains ‘backbone’ probes across the entire genome, and serves to normalize data across all probes. For each patient sample the data for only the gene(s) of interest is analyzed and reported.

FDA Notes
These results should be used in the context of available clinical findings, and should not be used as the sole basis for treatment. This test was developed and its performance characteristics determined by the PreventionGenetics Molecular Diagnostics Laboratories as required by the CLIA ‘88 regulations. It has not been cleared or approved for specific uses by the US Food and Drug Administration. This test is used for clinical purposes. It should not be regarded as investigational or for research.
Brief Description and Rationale: Array Comparative Genomic Hybridization (aCGH) enables the detection of deletion and duplications of single and multiple exons within a given gene (Tayeh et al. Genet Med 11:232-240, 2009). This Test involves analysis only of the specific gene(s) of interest for each patient. This Test is NOT a Chromosome Microarray (CMA) aCGH test.

PreventionGenetics' high density gene-centric (HDGC) aCGH is designed to have comprehensive coverage for both coding and non-coding regions for each targeted gene with very high density probe coverage. The average probe spacing within each exon is 47 bp or a minimum of three probes per exon covering all targeted exons and UTRs. The average probe spacing is 289 bp covering all intronic, 2kb upstream and downstream regions of each targeted gene. In addition, the flanking 300-bp intronic sequence on either side of targeted exons has enriched probe coverage. Therefore, PreventionGenetics' aCGH enables the detection of relatively small deletions and duplications within a single exon of a given gene or deletions and duplications encompassing the entire gene.

The frequency of deletions and duplications varies among genes, yet it represents a significant fraction of the total pathogenic mutations in essentially every gene (Tayeh et al. 2009). This fraction ranges from values as low as 5% (ACADM gene; Andressen et al. Am J Hum Genet. 68:1408-1418, 2001) up to 80% (NPHP1 gene; Konrad et al. Hum Mol Genet 5:367-371, 1996). In cases where the majority of the reported mutations in a gene can be detected by DNA sequencing, PreventionGenetics' aCGH is an excellent complementary test when DNA sequencing fails to identify the causative mutation(s). In cases where the majority of the reported mutations in a gene are deletions and duplications, then PreventionGenetics' aCGH should be considered as a primary test even before DNA sequencing. The availability of both DNA sequencing and PreventionGenetics' HDGC custom aCGH significantly improves the sensitivity of molecular clinical testing at PreventionGenetics.

Indications for Test: Candidates for this test are:
1. Patients with disorders where deletions and duplications contribute to the majority of the genetic burden.
2. Patients with autosomal dominant disorders where no mutation has been identified by DNA sequencing.
3. Patients with autosomal recessive disorders with one or no mutations have been identified by DNA sequencing.
4. Patients with autosomal recessive disorders where one or more amplicons within the gene fails to PCR amplify.
5. Male patients with X-linked disorders where no mutation has been identified by DNA sequencing, or PCR fails.
6. Female patients with X-linked disorders with one or no mutations have been identified by DNA sequencing.
7. Patients with gross genomic imbalances in a region harboring one or multiple genes targeted on PreventionGenetics' HDGC aCGH, to confirm involvement of such gene(s).

Sensitivity of Test: The prevalence of deletions and duplications vary among genes with an estimated range from near 0 to 80% (see [www.genetests.org](http://www.genetests.org)). PreventionGenetics' HDGC custom designed aCGH enables the detection of relatively small deletions and duplications within a single exon of a given gene or deletions and duplications encompassing the entire gene. Undocumented intronic and non-coding region deletions or duplications will require additional functional and/or family studies to determine clinical significance.

Limitations for reading-frame prediction: The reading frame predictions (in-frame or out-of-frame) for whole exon deletions / duplications are based on direct translation (in silico) of the mRNA ([see report for transcript analyzed](#)), which are generated by deletion / insertion (duplication) of the appropriate exons involved in this patient. Please note that any predictions derived from the analysis of DNA sequences may not correlate with the mRNA or protein sequences derived from such DNA sequences. In the absence of an experimental demonstration at the RNA or protein level, this prediction cannot be used as an evidence for changes at RNA or protein level. There are several reports of exceptions to the reading-frame rule, where changes at DNA level does not correspond exactly with changes at the RNA level (Aartsma-Rus et al. 2006). For example, more exons than those that are deleted might be missing at the RNA level because signals required for correct splicing are disrupted or deleted. In addition, intronic sequences flanked by inefficient splicing signals (so called 'cryptic' splice sites) might be activated yielding newly recognized exons incorporated in the mRNA.