Unconventional Diagnosis Based on Somatic Findings through Germ Line Whole-Exome Sequencing

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CASE DESCRIPTION

The proband is a 52-year-old man with a 2-year history of progressive symptoms including gait disturbance, hyperreflexia, muscle weakness, skeletal muscle atrophy, spasticity, and upper motor neuron dysfunction consistent with amyotrophic lateral sclerosis (ALS)[2]. Additionally, an abnormality of the cerebral white matter was seen on MRI. Whole-exome sequencing (WES) was performed on the proband and his unaffected mother and father in an attempt to identify a genetic cause for his ALS.

Variants were prioritized for manual review according to rarity (minor allele frequency <1% in population allele databases including Genome Aggregation Database, Exome Variant Server/Exome Sequencing Project, and 1000 Genomes), inheritance pattern (variants fitting de novo, autosomal recessive, and X-linked recessive patterns), and association with germ line diseases overlapping with the patient's clinical features. An investigation of potential candidate genes was also undertaken, prioritizing rare de novo variants in genes not known to be associated with underlying germ line disease. Sixteen variants were manually reviewed, of which 8 were noted to be de novo and with a variant allele frequency (VAF) <50%. After variant filtration and manual review of variants in genes associated with inherited disease, no variants thought likely to be causative of the patient's phenotype were identified. However, several variants flagged as de novo were present at VAFs suggestive of somatic (i.e., acquired) alterations. These variants included 2 variants in genes that had previously been associated with hematological neoplasms: NM_003016.4:c.284_307del (p.Pro95_Arg102del) in *SRSF2*[3] and NM_002168.3: c.419G>T (p.Arg140Leu) in *IDH2* (Table 1). Both variants were detected in roughly 20% of the patient's reads. Several other apparently somatic variants were observed at roughly the same VAF, although they were not reported because most were intronic and/or in genes of unclear relevance (data not shown). These incidental findings were only uncovered owing to our assessment of all de novo events.

On review of the patient's medical record, it was noted that a previous complete blood count showed decreased concentrations of leukocytes and neutrophils. Given the patient's age and absolute cytopenias, the presence of multiple somatic genetic abnormalities present at clonal allele frequencies was concerning for an underlying hematologic abnormality. There was no history of cancer or treatment for cancer. Of additional concern, the patient was considering enrollment for a clinical stem cell trial for ALS, of which exclusion criteria included the presence of underlying neoplasm(s). The patient had consented to receive secondary findings in accordance to American College of Medical Genetics' (ACMG's) list of 59 clinically relevant genes (*1*) but was not informed that uncovering additional clinically meaningful variants outside of this list was also possible. Thus, whether or not to disclose the incidental findings was, understandably, a topic of debate.

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Table 1. De novo variant findings SRSF2 and IDH2 variants identified in the patient, along with allele count and information available in publicly available databases.[a]

Gene (transcript)	Variant and genomic alteration	VAF (WES)	VAF Bone marrow (panel)	Overall GnomAD MAF[b] (v2.10.1)	HGMD Professional (v2019.1)	ClinVar	COSMIC (GRCh37, v88)	Classification
SRSF2 (NM_003016.4)	c.284_307del p.Pro95_Arg102del Chr17 (GRCh37): g.74732936_74732959del	24/130 (18%)	1051/3210 (52%)	0.00043% (1 allele seen in a 60–65-year-old individual of European, nonFinnish descent)	Absent	Absent	Hotspot for somatic changes. Variant reported in 79 samples of hematopoietic and lymphoid tissue.	Tier I level A (diagnostic/prognostic significance)
IDH2 (NM_002168.3)	c.419G>T p.Arg140Leu Chr15 (GRCh37): g.90631934C>A	24/127 (19%)	3793/9339 (41%)	Absent	Absent	pathogenic (4 submitters, no conflicts)	Hotspot for somatic changes. Variant reported in 19 samples of hematopoietic and lymphoid tissue. Variant at same codon (p.Arg140Gln) reported in 1298 samples.	Tier I (level A therapeutic significance)

[a] Variants were interpreted according to Association for Medical Pathology/American Society of Clinical Oncology/College of American Pathologists guidelines.
[b] MAF, minor allele frequency.

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QUESTIONS TO CONSIDER
• Why might molecular changes related to hematologic abnormalities be missed by whole-exome or whole-genome sequencing?
• When should reporting of incidental findings not included in the "ACMG 59" be strongly considered?
• How are we able to determine whether an alteration detected by WES is germ line or somatic?
• What might differences in the variant allele frequencies found in blood vs bone marrow specimens indicate?

References

1. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med 2017;19:249 –55.

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the January 2020 issue of *Clinical Chemistry*. To view the case and comments online, go to http://www.clinchem.org/content/vol66/issue1 and follow the link to the Clinical Case Study and Commentaries.

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