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SHORT REPORT

Juvenile myelomonocytic leukemia-associated variants are associated with neo-natal lethal Noonan syndrome

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Gain-of-function variants in some RAS–MAPK pathway genes, including *PTPN11* and *NRAS*, are associated with RASopathies and/or acquired hematological malignancies, most notably juvenile myelomonocytic leukemia (JMML). With rare exceptions, the spectrum of germline variants causing RASopathies does not overlap with the somatic variants identified in isolated JMML. Studies comparing these variants suggest a stronger gain-of-function activity in the JMML variants. As JMML variants have not been identified as germline defects and have a greater impact on protein function, it has been speculated that they would be embryonic lethal. Here we identified three variants, which have previously only been identified in isolated somatic JMML and other sporadic cancers, in four cases with a severe pre- or neo-natal lethal presentation of Noonan syndrome. These cases support the hypothesis that these stronger gain-of-function variants are rarely compatible with life.
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INTRODUCTION

Germline variants in RAS–MAPK pathway genes are associated with RASopathies, a genetically heterogeneous set of conditions whose clinical features include short stature, cardiovascular defects, development delays, characteristic facies, and skeletal, hematologic, and cutaneous findings.[1] Prenatal findings of RASopathies are nonspecific and include increased nuchal translucency, cystic hygroma, cardiac anomalies, and hydrops fetalis. Pathogenic variants in RAS–MAPK pathway genes have been identified in 9–17.3% of diploid fetuses with these ultrasound findings.[2–4]

Although germline variants in the RAS–MAPK pathway genes are associated with RASopathies, somatic variants in *PTPN11*, *NRAS*, *KRAS*, and *CBL* are initiating drivers for isolated juvenile myelomonocytic leukemia (JMML). JMML is an aggressive myeloproliferative neoplasm of early childhood that is commonly lethal without a hematopoietic stem cell transplant. Somatic variants in these genes are also observed, although less frequently, in other sporadic leukemias (eg, AML, ALL, and CMML) as secondary, cooperating variants in subclones.[5–7] Individuals with Noonan syndrome are at a high risk of developing a transient myeloproliferative disorder (MPD) during infancy, which resembles JMML but normally resolves without treatment, often referred to as JMML-like MPD.[1,8] However, JMML-like MPD can result in early lethality in a minority of individuals with RASopathies.[9]

Pathogenic variants in *PTPN11* or *NRAS* causing isolated JMML rarely overlap with those causing Noonan syndrome.[10–14] This has been attributed to the JMML variants having a stronger gain-of-function activity than RASopathy-associated variants. There are rare observations of Noonan-associated *PTPN11* variants identified in isolated JMML, and a few reported cases of germline inheritance of JMML variants.[4,14–16] These data have led to the suggestion that the strong gain-of-function variants observed in isolated JMML would be embryonic lethal if inherited as a germline variant.[2,13,14,17] Here, we present a case series supporting this model.

SUBJECTS AND METHODS

Case 1

On 15.2 weeks ultrasound, the fetus was found to have a cystic hygroma. At 17.4 weeks, a follow-up ultrasound identified a heart abnormality, pleural effusion, pericardial effusion, fetal hydrops, and persistent cystic hygroma. Prenatal testing demonstrated a normal karyotype and microarray. The pregnancy was terminated at 19 weeks gestation. The maternal and paternal ages at conception were 21 and 27 years, respectively.

Case 2

Prenatal ultrasonographic evaluation revealed a 9.0-mm nuchal translucency, cystic hygroma, and hydrops fetalis (Figure 1). Prenatal testing was negative for fetal infections and showed a normal karyotype and microarray. Following testing, the pregnancy was terminated. The maternal and paternal ages at conception were 26 and 32 years, respectively.

Case 3

On 12 weeks ultrasound, the fetus was found to have cystic hygroma. Non-immune hydrops fetalis, bilateral pleural effusions, lateral ventriculomegaly (left greater than right), polyhydramnios, absence of stomach bubble, absence of swallowing, hypertelorism, low-set ears, wide neck, mild retrognathia, and short limbs were identified on sequential ultrasound. The pregnancy resulted in a live birth at 33 weeks gestation. The postnatal period was complicated by thrombocytopenia, hypoxemia, bilateral pneumothoraces, and respiratory distress. A postnatal evaluation identified a normal karyotype, structurally and functionally normal heart, no evidence of esophageal atresia, and slightly below average limb length. On day of life 2, the neonate passed away because of

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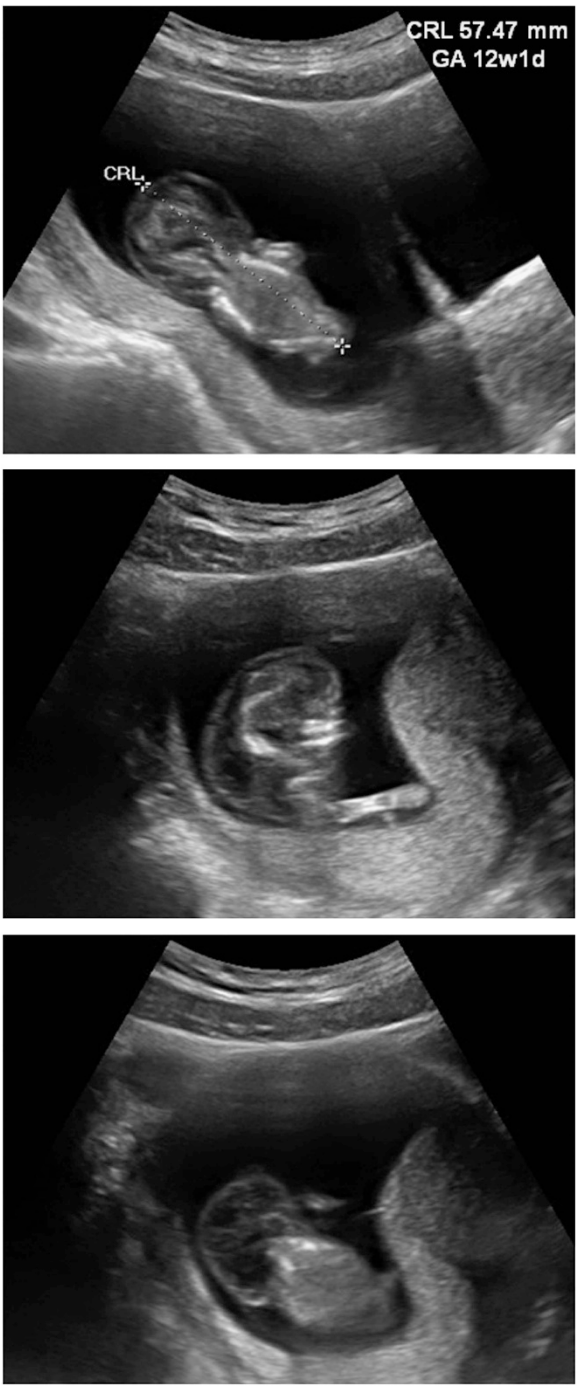

Figure 1 Ultrasonographic images at 12 weeks, 1 day from case 2 demonstrating the cystic hygroma.

pulmonary hypertension as a result of pulmonary hypoplasia secondary to non-immune fetal hydrops. The maternal and paternal ages at conception were 23 and 30 years, respectively.

Case 4

Prenatal ultrasonographic evaluation revealed a cystic hygroma. The pregnancy resulted in a live birth at 31 weeks gestation. The neonate had a low nasal bridge, hypertelorism, low-set posteriorly rotated ears, low hairline, webbed neck, thickened eyebrows, small upturned nose, short limbs, polydactyly of the left foot, coarseness, and scoliosis. The neonate was diagnosed with JMML by peripheral blood smear. On day of life 30, the neonate passed away from JMML and complications of necrotizing enterocolitis.

Variant analysis

DNA was extracted from amniocytes (cases 1 and 2), blood (case 3), or fibroblasts (case 4) using either Qiagen (Valencia, CA, USA) Puregene or Perkin Elmer (Waltham, MA, USA) Chemagen DNA extraction kits according to the manufacturers' recommendation. For cases 1–3, sequencing of *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1*, *MAP2K2*, *HRAS*, *SHOC2* exon 02, *CBL,* and *SPRED1* was performed by oligonucleotide-based target capture (SureSelect, Agilent, Santa Clara, CA, USA) and sequencing using Illumina HiSeq2000 instrument (50-base paired end; San Diego, CA, USA). Alignment and variant calls were performed as previously described using BWA and GATK (version 1.0.4705).[18] For case 4, a microarray-based resequencing assay (GeneChip, Affymetrix, Santa Clara, CA, USA) was used, as previously described.[19] For case 3, droplet digital PCR probes (ddPCR; Bio-Rad, Hercules, CA, USA) were used to quantitate variant fraction using the manufacturer's protocol. Sanger sequencing was used to fill in failed regions or sequenced regions with insufficient coverage (<20x), confirm clinically significant variants, and for parental testing of variants in *PTPN11* (NM_002834.3) or *NRAS* (NM_002524.3). Partners HealthCare Institutional Review Board approved this study. Variants were deposited in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar; SCV000204032, SCV000204031, and SCV000204071).

RESULTS

Sequencing of RAS–MAPK pathway genes in four cases with a severe pre- or neo-natal presentation of RASopathy identified three variants (Supplementary Figure 1) previously only reported as somatic changes in isolated JMML and other sporadic cancers. In case 1, c.227A>G (p.(Glu76Gly)) in exon 03 of *PTPN11* was identified (49% alternative allele fraction). Glu76 is a hotspot for somatic changes in JMML and p.(Glu76Gly) has been observed in >35 hematopoietic neoplasms, the majority being JMML.[20] In case 2, c.214G>A (p.(Ala72Thr)) in exon 03 of *PTPN11* was identified (51% alternative allele fraction). Ala72 is also a hotspot for JMML and p.(Ala72Thr) has been identified in >30 hematopoietic neoplasms, of which 15 were JMML.[20] In cases 3 and 4, c.34G>A (p.(Gly12Ser)) in exon 02 of *NRAS* was identified. In case 3, although the alternative allelic fraction from the NGS data (36%) was slightly low, mosaicism was excluded based upon ddPCR data (50% alternative allelic fraction). However, in case 4, mosaicism cannot be excluded as the variant was identified only via Sanger sequencing. Previous reports have described p.(Gly12Ser) in >75 hematopoietic neoplasms, the majority being AML.[20] In cases 1, 2, and 4, the variants were apparently *de novo* (paternity was not molecularly determined). Parental samples were not available for case 3.

DISCUSSION

Germline-inherited JMML variants in *PTPN11* have been hypothesized to be embryonic lethal because of their stronger gain-of-function activity and lack of reported germline observation.[2,13,14,17] Supporting this, a prior report described a pregnancy with a severe presentation including a 11-mm nuchal translucency, cystic hygroma, fetal hydrops, hydrothorax, and generalized skin edema with a *de novo* germline variant, c.227A>T (p.(Glu76Val)), typically seen in isolated JMML.[4] Similarly, another variant, c.1520C>A (p.(Thr507Lys)), seen exclusively in other leukemias, although not JMML, was identified in two fetuses with hydrops fetalis.[2,21] Our study lends further support to this hypothesis, given JMML variants were observed in cases 1 and 2, which both presented with severe prenatal abnormalities. However, as the pregnancy in the prior reports and two reported here were either

electively terminated or lost to follow-up, it remains suggestive that strong gain-of-function variants in *PTPN11* are incompatible with life.

Initial studies suggest that variants in *NRAS* have a similar spectrum as those in *PTPN11,* with mildly activating variants causing Noonan syndrome and strong gain-of-function activity variants acting as initiating drivers for isolated JMML.[10,11] Supporting this, most germline *NRAS* variants associated with Noonan syndrome (c.179G>A (p.(Gly60Glu)), c.71T>A (p.(Ile24Asn)), and c.149C>T (p.(Thr50Ile))) were found to be mildly activating when compared with the recurrent oncogenic variant, c.35G>T (p.(Gly12Val)).[10,11] In addition, embryonic expression of another oncogenic *NRAS* variant, p.(Gly12Asp), was embryonic lethal in mice.[22] Cases 3 and 4, harboring the c.34G>A (p.(Gly12Ser)) variant and resulting in early neo-natal death, support that germline-inherited oncogenic NRAS variants are embryonic lethal in humans.

Another oncogenic *NRAS* variant, c.38G>A (p.(Gly13Asp)), has been reported in two individuals without a severe RASopathy presentation. The first did not have any noted RASopathy features, but presented with infantile-onset leukemia and adult-onset hematological abnormalities,[15] suggesting this presentation is likely due to tissue-specific mosaicism. The second presented with an aggressive JMML-like MPD and, upon follow-up evaluation, features of a RASopathy.[16] Although further studies are necessary to determine if oncogenic *NRAS* variants result in early lethality, all reported cases with a germline-inherited oncogenic *NRAS* variant had a hematological abnormality. These observations suggest that there are variable phenotypes associated with germline inheritance of oncogenic *NRAS* variants, but these individuals are at risk for hematological abnormalities.

This study supports the model that JMML variants with germline inheritance result in severe prenatal and/or neo-natal presentation. Additional studies are required to determine if these *PTPN11* variants are embryonic lethal and if early death associated with these *NRAS* variants results from hematological abnormalities.

CONFLICT OF INTEREST

HM-S, DT, KAL, TEM and MSL are employed by non-profit, fee-for-service laboratories that offers genetic testing.

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1 Tartaglia M, Gelb BD: Disorders of dysregulated signal traffic through the RAS-MAPK pathway: phenotypic spectrum and molecular mechanisms. *Ann N Y Acad Sci* 2010; **1214**: 99–121.
2 Lee KA, Williams B, Roza K *et al*: PTPN11 analysis for the prenatal diagnosis of Noonan syndrome in fetuses with abnormal ultrasound findings. *Clin Genet* 2009; **75**: 190–194.
3 Houweling AC, de Mooij YM, van der Burgt I, Yntema HG, Lachmeijer AM, Go AT: Prenatal detection of Noonan syndrome by mutation analysis of the PTPN11 and the KRAS genes. *Prenat Diagn* 2010; **30**: 284–286.
4 Croonen EA, Nillesen WM, Stuurman KE *et al*: Prenatal diagnostic testing of the Noonan syndrome genes in fetuses with abnormal ultrasound findings. *Eur J Hum Genet* 2013; **21**: 936–942.
5 Miller CA, Wilson RK, Ley TJ: Genomic landscapes and clonality of *de novo* AML. *N Engl J Med* 2013; **369**: 1473.
6 Molteni CG, Te Kronnie G, Bicciato S *et al*: PTPN11 mutations in childhood acute lymphoblastic leukemia occur as a secondary event associated with high hyperdiploidy. *Leukemia* 2010; **24**: 232–235.
7 Ricci C, Fermo E, Corti S *et al*: RAS mutations contribute to evolution of chronic myelomonocytic leukemia to the proliferative variant. *Clin Cancer Res* 2010; **16**: 2246–2256.
8 Hasle H: Malignant diseases in Noonan syndrome and related disorders. *Horm Res* 2009; **72**(Suppl 2): 8–14.
9 Strullu M, Caye A, Lachenaud J *et al*: Juvenile myelomonocytic leukaemia and Noonan syndrome. *J Med Genet* 2014; **51**: 689–697.
10 Cirstea IC, Kutsche K, Dvorsky R *et al*: A restricted spectrum of NRAS mutations causes Noonan syndrome. *Nat Genet* 2010; **42**: 27–29.
11 Runtuwene V, van Eekelen M, Overvoorde J *et al*: Noonan syndrome gain-of-function mutations in NRAS cause zebrafish gastrulation defects. *Dis Model Mech* 2011; **4**: 393–399.
12 Bocchinfuso G, Stella L, Martinelli S *et al*: Structural and functional effects of disease-causing amino acid substitutions affecting residues Ala72 and Glu76 of the protein tyrosine phosphatase SHP-2. *Proteins* 2007; **66**: 963–974.
13 Chan RJ, Feng GS: PTPN11 is the first identified proto-oncogene that encodes a tyrosine phosphatase. *Blood* 2007; **109**: 862–867.
14 Tartaglia M, Martinelli S, Stella L *et al*: Diversity and functional consequences of germline and somatic PTPN11 mutations in human disease. *Am J Hum Genet* 2006; **78**: 279–290.
15 Oliveira JB, Bidere N, Niemela JE *et al*: NRAS mutation causes a human autoimmune lymphoproliferative syndrome. *Proc Natl Acad Sci USA* 2007; **104**: 8953–8958.
16 De Filippi P, Zecca M, Lisini D *et al*: Germ-line mutation of the NRAS gene may be responsible for the development of juvenile myelomonocytic leukaemia. *Br J Haematol* 2009; **147**: 706–709.
17 Tartaglia M, Niemeyer CM, Fragale A *et al*: Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. *Nat Genet* 2003; **34**: 148–150.
18 Pugh TJ, Kelly MA, Gowrisankar S *et al*: The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. *Genet Med* 2014; **16**: 601–608.
19 Kothiyal P, Cox S, Ebert J, Aronow BJ, Greinwald JH, Rehm HL: An overview of custom array sequencing. *Curr Protoc Hum Genet/editorial board, Jonathan L Haines [et al]* 2009; **Chapter 7**: Unit 7 17.
20 Forbes SA, Beare D, Gunasekaran P *et al*: COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* 2014; **43**: D805–D811.
21 Jain Ghai S, Keating S, Chitayat D: PTPN11 gene mutation associated with abnormal gonadal determination. *Am J Med Genet Pt A* 2011; **155A**: 1136–1139.
22 Wang J, Liu Y, Li Z *et al*: Endogenous oncogenic Nras mutation initiates hematopoietic malignancies in a dose- and cell type-dependent manner. *Blood* 2011; **118**: 368–379.

Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)