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Paternal Uniparental Disomy 11p15.5 in the Pancreatic Nodule of an Infant With Costello Syndrome: Shared Mechanism for Hyperinsulinemic Hypoglycemia in Neonates With Costello and Beckwith–Wiedemann Syndrome and Somatic Loss of Heterozygosity in Costello Syndrome Driving Clonal Expansion

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Abstract

Costello syndrome (CS) entails a cancer predisposition and is caused by activating *HRAS* mutations, typically arising de novo in the paternal germline. Hypoglycemia is common in CS neonates. A previously reported individual with the rare *HRAS* p.Gln22Lys had hyperinsulinemic hypoglycemia. Autopsy showed a discrete pancreatic nodule. The morphologic and immunohistochemistry findings, including loss of p57^{Kip2} protein, were identical to a focal lesion of congenital hyperinsulinism, however, no *KCNJ11* or *ABCC8* mutation was identified and germline derived DNA showed no alternation of the maternal or paternal 11p15 alleles. Here we report paternal uniparental disomy (pUPD) within the lesion, similar to the pUPD11p15.5 in Beckwith–Wiedemann syndrome (BWS). The similar extent of the pUPD suggests a similar mechanism driving hyperinsulinemia in both conditions. After coincidental somatic LOH and pUPD, the growth promoting effects of the paternally derived *HRAS* mutation, in combination with the increased function of the adjacent paternally expressed *IGF2*, may together result in clonal expansion. Although this somatic LOH within pancreatic tissue resulted in hyperinsulinism, similar LOH in mesenchymal cells may drive embryonal rhabdomyosarcoma (ERMS). Interestingly, biallelic *IGF2* expression has been linked to rhabdomyosarcoma tumorigenesis and pUPD11 occurred in all 8 ERMS samples from CS individuals. Somatic *KRAS* and *HRAS*

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mutations occur with comparable frequency in isolated malignancies. Yet, the malignancy risk in CS is notably higher than in Noonan syndrome with a *KRAS* mutation. It is conceivable that *HRAS* co-localization with *IGF2* and the combined effect of pUPD 11p15.5 on both genes contributes to the oncogenic potential.

Keywords

Costello syndrome; neonatal hyperinsulinemic hypoglycemia; loss of heterozygosity; Beckwith–Wiedemann syndrome; 11p15.5; imprinting; *HRAS* mutation; p.Q22K

INTRODUCTION

Costello syndrome is a rare autosomal dominant disorder caused by activating mutations in the Harvey rat sarcoma viral oncogene homolog (*HRAS*) [Aoki et al., 2005; Gripp et al., 2006; Kerr et al., 2006], typically arising de novo in the paternal germline [Sol-Church et al., 2006; Giannoulitou et al., 2013]. It is one of several syndromic conditions resulting from mutations affecting the RAS/MAPK pathway, collectively referred to as rasopathies. Costello syndrome affects all organ systems, including skin and craniofacial features, structural cardiovascular anomalies and hypertrophic cardiomyopathy [Lin et al., 2011], developmental delay/intellectual disability, and an increased risk for solid tumors with embryonal rhabdomyosarcoma being the most common [Gripp et al., 2002; Kerr et al., 2006]. The growth pattern is characterized by relatively high birth weight, followed by failure-to-thrive and short stature, with relative macrocephaly driven by cerebral and cerebellar overgrowth [Gripp et al., 2010; Krencik et al., 2015]. Feeding difficulties are typically severe and necessitate gastrostomy tube placement. Growth hormone deficiency is common, and may contribute to hypoglycemia in older individuals. Hypoglycemia is common in neonates and young infants with Costello syndrome [Gripp and Lin, 2012], occurring in 44% compared to 9% in Noonan and 6% of cardio-facio-cutaneous syndrome patients, respectively [Myers et al., 2014]. Although the mechanism for the neonatal hypoglycemia remains largely unknown, it typically resolves in early infancy. This course resembles that of neonatal hypoglycemia in Beckwith–Wiedemann syndrome, known to be associated with hyperinsulinism [Arnoux et al., 2012]. Hyperinsulinemic hypoglycemia was documented by Alexander et al. [2005] in two neonates with Costello syndrome and additionally in one with the extremely rare *HRAS* p.Gln22Lys mutation [Sheffield et al., 2015]. The latter individual developed medically intractable pulmonary hypertension and hypertrophic cardiomyopathy and died. Sheffield et al. [2015] identified a pancreatic nodule appearing morphologically identical to a focal lesion of congenital hyperinsulinism, but were unable to specify the underlying molecular change within the nodule. Here we report on further studies aiming at identification of that underlying molecular mechanism, showing paternal uniparental disomy (UPD) within the lesion, similar to the UPD11p15.5 seen in individuals with Beckwith–Wiedemann syndrome.

MATERIALS AND METHODS

The proband was reported by Sheffield et al. [2015] for his rare de novo *HRAS* mutation and his clinical course including hyperinsulinemic hypoglycemia. His physical findings were typical for Costello syndrome (Fig. 1). He developed medically intractable pulmonary hypertension and hypertrophic cardiomyopathy and died at age 13 weeks, at an adjusted age of 5 weeks given his premature delivery. The patient's DNA was extracted from the pancreatic nodule and from splenic tissue as previously reported [Sheffield et al., 2015]. The parents enrolled in an IRB approved research protocol and provided cheek swab DNA samples for analysis.

Mutation analysis was performed by PCR amplification of the *HRAS* exon 2 region using primers 5'-ACCTGTTCTGGAGGACGGTAA-3' and 5'-CCTCTAGAGGAAGCAGGAGACA-3'. Sequencing was performed in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). Short tandem repeat analysis (STR) was performed using the AmpFLSTR Identifiler PCR amplification Kit (Thermo Fisher Scientific) as well as 18 STR loci located along chromosome 11, originally designed by Applied Biosystems for the Linkage Mapping Set (LMS) MD10. The AmpFLSTR Identifiler PCR amplification Kit amplifies TH01 although D11S4046 and D11S1338 are part of the LMS. All LMS loci were purchased as custom primer sets and are labeled with either 6-FAM or VIC as the reporter dye. Sequencing and STR loci were both analyzed on an ABI3130xl Genetic Analyzer.

Parental origin of *HRAS* mutations in individuals with Costello syndrome was assessed using polymorphic markers as described in Sol-Church et al. [2006].

RESULTS

Sanger sequencing of *HRAS* showed the previously identified c.64 C>A (p.Gln22Lys), present in heterozygous state in the splenic tissue derived DNA, but skewed with the mutant allele accounting for more than 50% of all reads in the pancreatic nodule (Fig. 2). Skewing occurred for markers D11S4046 and TH01 (Fig. 3). No deviation from the expected heterozygous state was seen at D11S1338 (Fig. 3). Comparison to STR analysis of parental samples revealed an over-representation of the paternal alleles with loss of maternal alleles at D11S4046 and TH01 (Figs. 3 and 4).

In keeping with the previously reported de novo origin of the *HRAS* mutation based on peripheral white blood cell derived DNA [Sheffield et al., 2015], the parental cheek swab derived DNA samples did not show the *HRAS* mutation (data not shown). Due to the absence of informative intragenic markers we cannot show directly that the *HRAS* mutation occurred on the paternal allele.

As part of our ongoing research on Costello syndrome, parental origin of apparent de novo *HRAS* mutations resulting in a phenotype consistent with Costello syndrome was informative for 78 individuals, in 74 paternal origin was documented. Two of the four maternally derived mutations were seen in siblings [Gripp et al., 2011], implying maternal

gonadal mosaicism. Thus, at most, 2/78 (2.5%) were de novo mutations arising in the maternal germline.

DISCUSSION

The patient reported by Sheffield et al. [2015] had a rare de novo *HRAS* mutation and his clinical course included hyperinsulinemic hypoglycemia. He died at age 13 weeks, at an adjusted age of 5 weeks given his premature delivery. Upon autopsy, a discrete pancreatic nodule was identified. The morphologic and immunohistochemistry findings, including loss of p57^{Kip2} protein, were identical to a focal lesion of congenital hyperinsulinism [Sheffield et al., 2015], however, no mutation in *KCNJ11* or *ABCC8* was identified in DNA extracted from the nodule. Studies of germline derived DNA indicated no alterations to the maternal or paternal alleles at 11p15. Here we performed further molecular studies on the DNA derived from the nodule, revealing LOH of 11p15.5 but not extending beyond 11p15.4. The lack of p57^{Kip2} expression within the nodule strongly suggests that the maternal allele is lost, and this was confirmed through STR analysis (Fig. 3). We identified an overrepresentation of the mutated *HRAS* allele, above the expected 50%, in the DNA sample derived from the pancreatic lesion, but not in the splenic tissue (Fig. 2). In addition to skewing at the mutation site, allelic imbalance leading to partial loss of heterozygosity (LOH) was observed for additional markers in 11p15.5 (Fig. 3). This LOH was due to loss of the maternal alleles (Fig. 3). D11S1338 located at 11p15.4 (Fig. 4) as well as markers located on the q arm showed no allelic imbalance. These results demonstrate LOH for distal 11p within the pancreatic nodule, incomplete likely due to contamination by non-neoplastic cells. Due to the absence of discriminating SNPs within the *HRAS* alleles, we cannot show directly that the de novo mutation arose in the paternal germline. However, based on the high mutation rate for *HRAS* documented in the paternal germline [Giannoulitou et al., 2013] and the resulting overrepresentation of paternally derived *HRAS* mutations reported here, it is highly likely that this mutation arose in the paternal germline. Further, the allelic imbalance within the pancreatic lesion favors the paternal STR alleles (Fig. 3) as well as the mutant *HRAS* allele (Fig. 2), suggesting that the mutation occurred on the paternal chromosome 11. The related loss of the maternally derived distal 11p (Figs. 3 and 4) explains the observed lack of p57^{KIP2} protein within the pancreatic nodule [Sheffield et al., 2015], because *CDKN1C* encoding p57^{KIP2} is exclusively maternally expressed.

Hypoglycemia in Beckwith–Wiedemann Syndrome

Loss of heterozygosity at 11p15.5 with resulting paternal isodisomy and loss of the maternal allele accounts for 20% of Beckwith–Wiedemann syndrome cases, with other mechanisms affecting imprinted gene expression at 11p15.5 in the remainder [Shuman et al., 2000]. The abnormal expression of imprinted genes is associated with numerous physical abnormalities and understanding of each gene's respective contribution to the phenotype remains incomplete. About 50% of neonates with Beckwith–Wiedemann syndrome show hypoglycemia, typically due to hyperinsulinism [Hussain et al., 2005] resolving within the first weeks of life [Arnoux et al., 2012]. Of note, an individual with multiple congenital anomalies due to a paternally derived duplication 11p15.5p11.12 showed hyperinsulinemic

hypoglycemia, consistent with the hypothesis that dosage skewing towards the paternal allele drives hyperinsulinemia [Gardeitchik et al., 2012].

Hypoglycemia in Costello Syndrome

Interestingly, compared to Beckwith–Wiedemann syndrome, hypoglycemia is almost as common in neonates and young infants with Costello syndrome, occurring in 44% compared to only 9% in Noonan and 6% of cardio-facio-cutaneous syndrome patients, respectively [Myers et al., 2014]. Although the underlying mechanism for neonatal hypoglycemia in Costello syndrome often remains unclear, hyperinsulinemia has occasionally been documented [Alexander et al., 2005]. In an individual with the rare *HRAS* p.Gly12Glu mutation, severe neonatal hypoglycemia due to congenital hyperinsulinism was associated with hypertrophy and hyperplasia of Langerhans islets seen on autopsy [Case 11 in Kerr et al., 2006]. Neonatal hypoglycemia in Costello syndrome is typically responsive to diazoxide treatment [Alexander et al., 2005] or resolves after a brief period of intervention and glucose level monitoring. This course is notably similar to that seen in Beckwith–Wiedemann syndrome, and differs from that of patients with focal hyperinsulinism.

Loss-Of-Heterozygosity at 11p in Hypoglycemia

Two distinct mechanisms involving LOH at 11p cause hyperinsulinemic hypoglycemia. Distal pUPD results in absence of *p57^{KIP2}*, encoded by the exclusively maternally expressed *CDKN1C*, and is present in about 20% of individuals with Beckwith–Wiedemann syndrome, where the abnormal expression of imprinted genes in this region is the underlying cause for multiple physical differences including hyperinsulinism. Here we report pUPD of the same region (Fig. 4) in the pancreatic endocrine cell proliferation of an individual with Costello syndrome and hyperinsulinemic hypoglycemia.

The second mechanism affects LOH extending further centromerically and is seen in focal hyperinsulinism. Focal hyperinsulinism is caused by a paternally inherited ATP-sensitive potassium channel mutations in the biallelic expressed *KCNJ11* or *ABCC8*, together with a somatic loss of maternal 11p15 [Suchi et al., 2012] and pUPD (Fig. 5). *KCNJ11* and *ABCC8* are located at 11p15.1, centromeric to *HRAS* and *CDKN1C* at 11p15.5 and 11p15.4, respectively. The paternally inherited *KCNJ11* or *ABCC8* mutation is present in all cells, whereas the secondary sporadic occurrence of LOH in pancreatic tissue accounts for the focal nature of the pancreatic lesion. The clinical course of focal hyperinsulinism is characterized by its unremitting nature and resistance to diazoxide treatment [Glaser, 2003], thus contrasting to the typical course of hypoglycemia in Beckwith–Wiedemann and Costello syndrome. No mutation in *KCNJ11* or *ABCC8* was found in the patient with Costello syndrome and hyperinsulinemia [Sheffield et al., 2015], and our results showed that the pUPD was limited to distal 11p (Figs. 3 and 4).

Loss-Of-Heterozygosity in Costello Syndrome

Paternal UPD for distal 11p in the pancreatic nodule of the individual with Costello syndrome resulted from the somatic LOH in the pancreatic tissue (Fig. 5). Given the similar extent of the paternal UPD in some individuals with Beckwith–Wiedemann syndrome and their similar clinical course, it appears likely that the same mechanism drives the

hyperinsulinemia. The paternally expressed *IGF2*, located just telomeric to the maternally expressed *CDKN1C*, may play a significant role. It exerts a growth promoting effect in embryonic and fetal development, mirroring the hypoglycemia most prominent in the neonatal and early infant ages. Although the reported somatic LOH within pancreatic tissue resulted in hypoglycemia, similar LOH may occur in mesenchymal cells giving rise to myoblasts (Fig. 5), allowing for development of embryonal rhabdomyosarcoma (ERMS), the most common malignancy in Costello syndrome. Indeed, paternal UPD11 occurred in all eight ERMS samples from Costello syndrome individuals [Robbins et al., 2015] and has been reported in nonsyndromic ERMS [Chen et al., 2015]. Uniparental disomy 11p was previously noted in Costello syndrome related ERMS [Kerr et al., 2003]. Kratz et al. [2007] found UPD at 11p15.5 in 5/6 nonsyndromic ERMS, and two samples harbored a heterozygous *HRAS* mutation. The heterozygosity of the *HRAS* mutations implied that they arose after the LOH and the authors concluded that “imbalance at 11p15.5 and *HRAS* mutations represent independent but cooperating events during ERMS development.” Parent-of-origin information was not available for these samples [Kratz et al., 2007] and the Costello syndrome ERMS studied by Kerr et al. [2003], but paternal UPD may be suspected based on information provided in this report and elsewhere [Chen et al., 2015; Robbins et al., 2015]. Interestingly, biallelic *IGF2* expression has been linked to rhabdomyosarcoma tumorigenesis. It is possible that the growth promoting effects of the Costello syndrome causing *HRAS* mutation, which happens to be paternally derived in most cases, in combination with the increased signaling function of the paternally expressed *IGF2* after coincidental somatic LOH, together result in the potential for clonal expansion. If the somatic LOH occurs in pancreatic tissue the focal lesion may cause hyperinsulinism, whereas such clonal expansion in mesenchymal cells may result in an ERMS. One may speculate that the same mechanism occurring in the precursor for the adrenal cells causes neuroblastoma, another tumor seen with increased frequency in Costello syndrome.

Allelic Imbalance in *Hras* and *HRAS* Related Tumors

In a *Hras*^{G12V} knock-in mouse model of Costello syndrome developing skin papillomata, amplification of the mutant allele occurred only at the papilloma stage [Chen et al., 2009, 2014]. Angiosarcoma and forestomach papillomata showed similar increase in the copy number of the mutant allele [Chen et al., 2009], leading to the conclusion that further augmentation of the signal output from a single oncogenic *Hras* mutation is required for tumor development. *HRAS* allelic imbalance favoring the mutant allele was observed in human thyroid cancer cell lines, supporting this conclusion [Chen et al., 2014].

CONCLUSIONS

Discovery of LOH within the pancreatic lesion of an individual with Costello syndrome is important not only to understand commonly seen neonatal hypoglycemia in such patients, but additionally it highlights LOH with pUPD as a mechanism resulting in dysregulated cell growth and tumorigenesis. This mechanism is likely relevant not only to hyperinsulinism, but also for tumor and papillomata development in Costello syndrome. Somatic mutations in *KRAS* and *HRAS* occur with comparable frequencies in isolated malignancies. Yet, the risk for malignancies in Costello syndrome is notably higher compared to other rasopathies,

including Noonan syndrome due to mutations in *KRAS*. Although this difference could be due to the differential effect and function of *HRAS* compared to *KRAS*, it is conceivable that the co-localization with *IGF2* and the combined effect of paternal UPD for 11p15.5 on both genes contributes to the oncogenic potential.

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REFERENCES

- Alexander S, Ramadan D, Alkhayat H, Al-Sharkawi I, Aboo Backer KC, El-Sabban F, Hussain K. Costello syndrome and hyperinsulinemic hypoglycemia. *Am J Med Genet Part A*. 2005; 139A:227–230. [PubMed: 16278907]
- Aoki Y, Niihori T, Kawame H, Kurosawa K, Ohashi H, Tanaka Y, Filocamo M, Kato K, Suzuki Y, Kure S, Matsubara Y. Germline mutations in *HRAS* proto-oncogene cause Costello syndrome. *Nat Genet*. 2005; 37:1038–1040. [PubMed: 16170316]
- Arnoux, JB.; Verkarre, V.; Rossignol, S.; Aigrain, Y.; de Lonlay, P. Molecular mechanisms and clinical pathophysiologies of focal ATP-sensitive potassium channel hyperinsulinism and Beckwith–Wiedemann syndrome.. In: Stanley, CA.; Leon de, editors. *Monogenic hyperinsulinemic hypoglycemia disorders*. Front diabetes. Vol. 21. Basel; Karger AG: 2012. p. 43-56.
- Chen L, Shern JF, Wei JS, Yohe ME, Song YK, Hurd L, Liao H, Catchpoole D, Skapek SX, Barr FG, Hawkins DS, Khan J. Clonality and evolutionary history of rhabdomyosarcoma. *PLoS Genet*. 2015; 11:e1005075. [PubMed: 25768946]
- Chen X, Makarewicz JM, Knauf JA, Johnson LK, Fagin JA. Transformation by *Hras*G12V is consistently associated with mutant allele copy gains and is reversed by farnesyl transferase inhibition. *Oncogene*. 2014; 33:5442–5449. [PubMed: 24240680]
- Chen X, Mitsutake N, LaPerle K, Akeno N, Zanzonico P, Longo VA, Mitsutake S, Kimura ET, Geiger H, Santos E, Wendel HG, Franco A, Knauf JA, Fagin JA. Endogenous expression of *Hras* G12V induces developmental defects and neoplasms with copy number imbalances of the oncogene. *PNAS*. 2009; 106:7979–7984. [PubMed: 19416908]
- Gardeitchik T, de Leeuw N, Nijtmans L, Jira P, Kozicz T, Czako M, van de Burgt I, Morava E. Infant with MCA and severe cutis laxa due to a de novo duplication 11p of paternal origin. *Am J Med Genet A*. 2012; 158A:469–472. [PubMed: 22173889]
- Giannoulatou E, McVean G, Taylor IB, McGowan SJ, Maher GJ, Iqbal Z, Pfeifer SP, Turner I, Burkitt Wright EM, Shorto J, Itani A, Turner K, Gregory L, Buck D, Rajpert-De Meyts E, Looijenga LH, Kerr B, Wilkie AO, Goriely A. Contributions of intrinsic mutation rate and selfish selection to levels of de novo *HRAS* mutations in the paternal germline. *Proc Natl Acad Sci USA*. 2013; 110:20152–20157. [PubMed: 24259709]
- Glaser, B. Familial hyperinsulinism.. In: Pagon, RA.; Adam, MP.; Ardinger, HH., editors. *GeneReviews*® [Internet].. University of Washington, Seattle; Seattle (WA): 2003. 1993-2015. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK1375/>
- Gripp KW, Lin AE, Stabley DL, Nicholson L, Charles I, Scott CI Jr, Doyle D, Aoki Y, Matsubara Y, Zackai EH, Lapunzina P, Gonzalez-Meneses A, Holbrook J, Agresta CA, Gonzalez IL, Sol-Church K. *HRAS* mutation analysis in Costello syndrome: Genotype and phenotype correlation. *Am J Med Genet*. 2006; 140A:1–7. [PubMed: 16329078]
- Gripp KW, Hopkins E, Doyle D, Dobyns WB. High incidence of progressive postnatal cerebellar enlargement in Costello syndrome: Brain overgrowth associated with *HRAS* mutations as the likely cause of structural brain and spinal cord abnormalities. *Am J Med Genet Part A*. 2010; 152A:1161–1168. [PubMed: 20425820]

- Gripp KW, Scott CI Jr, Nicholson L, McDonald-Ginn DM, Ozeran JD, Lin AE, Zackai EH. Five additional Costello syndrome patients with rhabdomyosarcoma: Proposal for a tumor screening protocol. *Am J Med Genet.* 2002; 108:80–87. [PubMed: 11857556]
- Gripp KW, Lin AE. Costello syndrome: A Ras/mitogen activated protein kinase pathway syndrome (rasopathy) resulting from HRAS germline mutations. *Genet Med.* 2012; 14:285–292. [PubMed: 22261753]
- Gripp KW, Stabley DL, Geller PL, Hopkins E, Stevenson DA, Carey JC, Sol-Church K. Molecular confirmation of HRAS p.G12S in siblings with Costello syndrome. *Am J Med Genet Part A.* 2011; 155:2263–2268. [PubMed: 21834037]
- Hussain K, Cosgrove KE, Shepherd RM, Luharia A, Smith VV, Kassem S, Gregory JW, Sivaprasadarao A, Christesen HT, Jacobsen BB, Brusgaard K, Glaser B, Maher EA, Lindley KJ, Hindmarsh P, Dattani M, Dunne MJ. Hyperinsulinemic hypoglycemia in Beckwith–Wiedemann syndrome due to defects in the function of pancreatic beta-cell adenosine triphosphate-sensitive potassium channels. *J Clin Endocrinol Metab.* 2005; 90:4376–4382. [PubMed: 15811927]
- Kerr B, Delrue MA, Sigaudy S, Perveen R, Marche M, Burgelin I, Stef M, Tang B, Eden OB, O'Sullivan J, De Sandre-Giovannoli A, Reardon W, Brewer C, Bennett C, Quarell O, M'Cann E, Donnai D, Stewart F, Hennekam R, Cave H, Verloes A, Philip N, Lacombe D, Levy N, Arveiler B, Black G. Genotype-phenotype correlation in Costello syndrome: HRAS mutation analysis in 43 cases. *J Med Genet.* 2006; 43:401–405. [PubMed: 16443854]
- Kerr B, Muchielli ML, Sigaudy S, Fabre M, Saunier P, Voelckel MA, Howard E, Elles R, Eden TOB, Black GC, Philip N. Is the locus for Costello syndrome on 11p? *J Med Gene.* 2003; 40:469–471.
- Kratz CP, Steinemann D, Niemeyer CM, Schlegelberger B, Koscielniak E, Kontny U, Zenker M. Uniparental disomy at chromosome 11p15. 5 followed by HRAS mutations in embryonal rhabdomyosarcoma: Lessons from Costello syndrome. *Hum Molec Genet.* 2007; 16:374–379. [PubMed: 17164262]
- Krencik R, Hokanson KC, Narayan AR, Dvornick J, Rooney GE, Rauen K, Weiss LA, Rowitch DH, Ullian EM. Dysregulation of astrocyte extracellular signaling in Costello syndrome. *Sci Transl Med.* 2015; 7:286ra66.
- Lin AE, Alexander ME, Colan SD, Kerr B, Rauen KA, Noonan J, Baffa J, Hopkins E, Sol-Church K, Limongelli G, Digilio MC, Marino B, Ines AM, Aoki Y, Silberbach M, Del-Rue MA, While SM, Hamilton RM, O'Connor W, Grossfeld PD, Smoot LB, Padera RF, Gripp KW. Clinical, pathological and molecular analyses of cardiovascular abnormalities in Costello syndrome: A Ras/MAPK Pathway syndrome. *Am J Med Genet Part A.* 2011; 155A:486–507. [PubMed: 21344638]
- Myers A, Bernstein JA, Brennan ML, Curry C, Esplin ED, Fisher J, Homeyer M, Manning MA, Muller EA, Niemi AK, Seaver LH, Hintz SR, Hudgins L. Perinatal features of the RASopathies: Noonan syndrome, Cardiofaciocutaneous syndrome and Costello syndrome. *Am J Med Genet Part A.* 2014; 164A:2814–2821. [PubMed: 25250515]
- Robbins, KM.; Stabley, DL.; Holbrook, J.; Sahraoui, RJ.; Gripp, KW.; Sol-Church, K. Using a Costello syndrome cohort as a syndromic model to better understand embryonal rhabdomyosarcoma at the molecular, cytogenetic, and functional level.. (#2793W) presented at the 65th annual meeting of the American society of human genetics; Baltimore, MD.. October 7, 2015; 2015.
- Sheffield BS, Yip S, Ruchelli ED, Dunham CP, Sherwin E, Brooks PA, Sur A, Singh A, Human DG, Patel MS, Lee AF. Fatal congenital hypertrophic cardiomyopathy and a pancreatic nodule morphologically identical to focal lesion of congenital hyperinsulinism in an infant with Costello syndrome: Case report and review of the literature. *Pediatr Dev Pathol.* 2015; 18:237–244. [PubMed: 25668678]
- Shuman, C.; Beckwith, JB.; Smith, AC.; Weksberg, R. Beckwith–Wiedemann syndrome.. In: Pagon, RA.; Adam, MP.; Ardinger, HH., editors. *GeneReviews*® [Internet].. University of Washington, Seattle; Seattle (WA): 2000. 1993-2015. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK1394/>
- Sol-Church K, Stabley DL, Nicholson L, Gonzalez IL, Gripp KW. Paternal bias in parental origin of HRAS mutations in Costello syndrome. *Hum Mutat.* 2006; 27:736–741. [PubMed: 16835863]

Suchi, M.; Bhatti, TR.; Ruchelli, E. Pancreatic histopathology of hyperinsulinism.. In: Stanley, CA.; de Leon, DD., editors. Monogenic hyperinsulinemic hypoglycemia disorders. Front Diabetes. Vol. 21. Basel; Karger: 2012. p. 57-70.

FIG. 1.

Proband as neonate, note hydropic appearance, prominent philtrum, and lips. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>].

Proband *HRAS* mutation status
CAG>AAG (p.Gln22Lys)

FIG. 2.

Sanger sequencing of *HRAS* showed the previously identified c.64 C>A (p.Gln22Lys), present in heterozygous state in the splenic tissue derived DNA, but skewed in the pancreatic nodule. Sequencing in both directions shows an increase of the mutant allele over the wild-type. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>].

Chromosome 11 STR Status

FIG. 3.

Fragment analysis of chromosome 11 short tandem repeat (STR) markers shows heterozygous amplification in normal splenic tissue and allelic imbalance in the pancreatic nodule for markers D11S4046 and TH01. No deviation from the expected heterozygous state was seen at D11S1338. Parental results show the partial loss of heterozygosity in the nodule is due to paternal uniparental disomy. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>].

FIG. 4.

Genomic location of markers used to define the LOH in the pancreatic nodule. Skewing from a normal heterozygous state towards paternal UPD occurred in genomic DNA at three loci within 11p15.5: The mutation site within *HRAS* and STR loci D11S4046 and TH01. Loss of the protein p57KIP2, encoded by the maternally expressed *CDKN1C*, supports LOH into 11p15.4, but not past STR locus D11S1338 which was not skewed. All other STR loci centromeric to D11S1338 and on 11q were normal heterozygous. Positions are NC_000011.10. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>].

FIG. 5.

Diagram of UPD for chromosome 11 and of point mutations affecting either *ABCC8* or *HRAS*, as seen in Beckwith–Wiedemann syndrome, focal familial hyperinsulinism or Costello syndrome, respectively. Somatic loss of heterozygosity in the respective tissue may result in clonal expansion. [Color figure can be seen in the online version of this article, available at <http://wileyonlinelibrary.com/journal/ajmga>].