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Craniosynostosis and Noonan Syndrome with *KRAS* Mutations: Expanding the Phenotype with a Case Report and Review of the Literature

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Noonan syndrome (NS) is a multiple congenital anomaly syndrome caused by germline mutations in genes coding for components of the Ras-mitogen-activated protein kinase (RAS-MAPK) pathway. Features include short stature, characteristic facies, congenital heart anomalies, and developmental delay. While there is considerable clinical heterogeneity in NS, craniosynostosis is not a common feature of the condition. Here, we report on a 2 month-old girl with Noonan syndrome associated with a de novo mutation in *KRAS* (p.P34Q) and premature closure of the sagittal suture. We provide a review of the literature of germline *KRAS* mutations and find that approximately 10% of published cases have craniosynostosis. Our findings expand on the NS phenotype and suggest that germline mutations in the *KRAS* gene are causally involved in craniosynostosis, supporting the role of the RAS-MAPK pathway as a mediator of aberrant bone growth in cranial sutures. The inclusion of craniosynostosis as a possible phenotype in *KRAS*-associated Noonan Syndrome has implications in the differential diagnosis and surgical management of individuals with craniosynostosis. © 2015 Wiley Periodicals, Inc.

Key words: Noonan syndrome; *KRAS*; craniosynostosis; RAS-MAPK; RASopathy

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INTRODUCTION

Noonan syndrome (NS) is an autosomal dominant multiple congenital anomaly disorder with an estimated prevalence of 1/1,000–1/2,500 births [Romano et al., 2010]. Features of NS include short stature, characteristic facies, congenital heart malformations, as well as developmental delay [Allanson, 2007]. An important diagnostic trait in NS, facial features include widely spaced eyes, depressed nasal root, a deeply grooved philtrum, and low-set ears; these features are most noticeable during infancy and childhood. Birth weight and length are normal, but growth failure usually becomes apparent during the first year of life [Otten and Noordam, 2009]. Additional clinical findings include bleeding problems, sternal malformations, renal anomalies, gastrointestinal and feeding issues, lymphatic issues, cryptorchidism, as well as oral and dental issues.

Ten genes have been found to cause Noonan syndrome [Allanson and Roberts 2001; Aoki et al., 2013; Rauen, 2013]. Mutations in *PTPN11* are the most common cause of NS and account for about half [Tartaglia et al., 2001]. *PTPN11* is part of the RAS-MAPK (mitogen-activated protein kinase) signal transduction pathway, which has component genes that cause a group of developmental disorders with overlapping phenotypic features. This clinically defined group of medical genetic syndromes is known as the RASopathies. Mutations in *KRAS*, another RAS-MAPK gene, account for approximately 3% of NS cases and 7% of all RASopathies including cardio-facio-cutaneous syndrome (CFC) and a few individuals with a phenotype suggesting Costello syndrome (CS) [Brasil et al., 2012]. Due to variable expressivity and ascertainment bias, the penetrance of

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NS is unknown and many adults are only diagnosed after having an affected child [Allanson et al., 2001].

Craniosynostosis occurs in approximately 1 in 3,000 live births and is characterized by the premature fusion of one or more cranial sutures resulting in malformation of the skull. Potential consequences of the abnormal skull growth include increased intracranial pressure, problems with hearing and vision, impaired blood flow in the cerebrum, as well as developmental delay [Cunningham et al., 2007]. Craniosynostosis mostly occurs as an isolated and sporadic anomaly in an otherwise normal child, but may be found in over 150 described syndromes [Boulet et al., 2008]. The most common craniosynostosis syndromes include Apert syndrome, Crouzon syndrome, Saethre–Chotzen syndrome, and Muenke syndrome.

Despite the clinical heterogeneity of NS, craniosynostosis is not a recognized feature with very few cases reported. Here, we further expand on the clinical spectrum of NS with a case report and a review of the literature on craniosynostosis in NS as well as other RASopathies. We report a on a 2 month-old girl with NS due to a de novo mutation in *KRAS* and premature closure of the sagittal suture.

MATERIALS AND METHODS

The research protocol was approved by the National Human Genome Research Institute (NHGRI) Institutional Review Board. Informed consent was obtained from the parents for molecular analysis after which venous blood was drawn from the proband and her parents.

DNA Extraction

Genomic DNA was extracted from blood samples using the QIAamp DNA Blood Maxi Kit (QIAGEN, Valencia, CA). DNA samples were further prepared for next-generation sequencing by phenol/chloroform extraction.

Next-Generation Sequencing

Exome sequencing was performed by the National Intramural Sequencing Center (NISC); assembly, genotyping, and annotation of the proband and parents were performed as previously described [Johnston et al., 2010; Teer et al., 2010]. The NimbleGen SeqCap EZ Version 3.0 + UTR (Roche NimbleGen, Madison, WI) was used for capture, totaling approximately 96 Mb. The HiSeq2000 Sequencer protocol (Illumina, San Diego, CA) was followed for flow cell preparation and 125 bp paired end read sequencing. The percentage of the Consensus Coding Sequence exome with most probable genotype quality scores of 10 exceeded 85%. Variant list evaluation was performed using VarSifter [Teer et al., 2012].

Sanger Sequence Analysis

Sequence verification was performed using standard methods [Sanger et al., 1977] with v3.1 BigDye Terminator Cycle Sequencing Kit (Life Technologies, Grand Island, NY) in the ABI 3730xl Sequencer (Life Technologies). Alignment to the published reference genomic sequence was done with Sequencher 5.0.1 (Gene Codes Corp., Ann Arbor, MI).

Literature Review

A Medline search was conducted to find previously reported patients with *KRAS*-associated RASopathies and craniosynostosis. The key words and search terms used included: *KRAS*, Noonan syndrome, craniosynostosis, Costello syndrome, cardio-facio-cutaneous syndrome, RASopathies. References were also obtained from papers found through the literature search. Further literature search was done to find patients with craniosynostosis and RASopathies caused by other RAS-MAPK genes.

Clinical Report

The proband, a female, was born as the first child of a non-consanguineous Indian couple. Thickened nuchal translucency was noted during prenatal ultrasound, with a subsequent karyotype showing 46,XX. No prenatal or birth complications were noted. The patient presented for clinical evaluation at two months of age after her parents were concerned about dysmorphic facial features (Fig. 1). Craniofacial anomalies included a tall forehead, hypertelorism with a left down slanting palpebral fissure, bilateral ptosis and low-set ears with thick helices. The patient's philtrum was noted to be long and deep, the hair was sparse around the fronto-temporal region, and there was hypotrichosis of the eyebrows. The patient was noted to have broad great toes suggestive of a craniosynostosis syndrome (Fig. 2). Cranial three-dimensional computed tomography of the patient revealed sagittal suture synostosis (Fig. 3). Echocardiography and abdominal ultrasound were normal. The diagnosis of Noonan syndrome was initially masked by the patient's craniosysnostosis and broad toes, but was made later after exome sequencing revealed a mutation in *KRAS*.

RESULTS

Exome Sequencing

Using whole exome sequencing, a de novo heterozygous mutation was found in *KRAS*: c.101C>A; p.P34Q. This mutation was reported in a

FIG. 1. Facial features significant for hypertelorism and down slanting palpebral fissures left down slanting palpebral fissure, bilateral ptosis, long and deep philtrum, sparse hair around the fronto-temporal region, and hypotrichosis of the eyebrows.

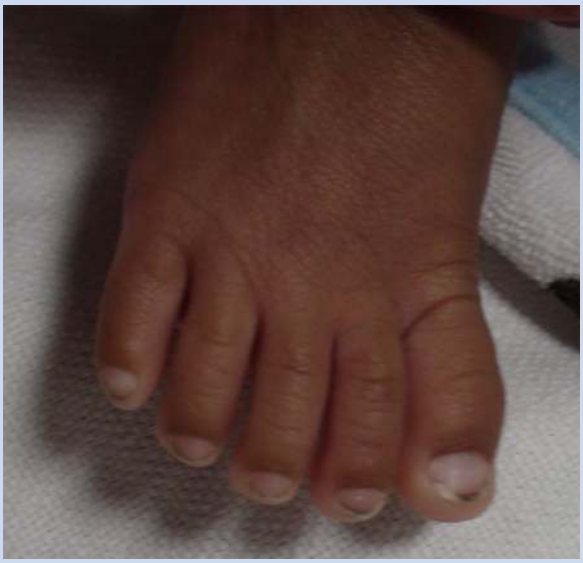

FIG. 2. Broad right great toe.

3-year-old boy without craniosynostosis diagnosed with Noonan syndrome [Zenker et al., 2007]. This variant occurs in a conserved amino acid in the G domain of *KRAS* [Gremer et al., 2011]. The mutation was verified by Sanger sequencing in the proband but was absent in both parents. Multiple prediction models were used to evaluate the potential pathogenicity, including a Combined Annotation-Dependent Depletion (CADD) score of 27.3, a Grantham score of 76, a genomic evolutionary rate profiling (GERP) score of 5.7, and a PolyPhen-2 (Polymorphism Phenotyping v2) prediction of "probably damaging." Exome sequencing revealed no pathogenic variants in genes that cause the common craniosynostosis syndromes including *FGFR1, FGFR2, FGFR3, TWIST1, MSX2*, and *EFNB1*.

Literature Search

A total of 61 reported cases of RASopathies with *KRAS* mutations were identified in the literature (See Supplementary Table S1) [Carta et al., 2006; Niihori et al., 2006; Schubbert et al., 2006; Bertola et al., 2007; Nava et al., 2007; Sovik et al., 2007; Zenker et al., 2007; Ko et al., 2008; Lo et al., 2008; Nystrom et al., 2008; Kratz et al., 2009; Leventopoulos et al., 2010; Adaci et al., 2012; Brasil et al., 2012; Choi et al., 2012; Malaquias et al., 2012; Ortiz et al., 2012; Stark et al., 2012; Cronnen et al., 2013; Nosun et al., 2013; Quaio et al., 2013; Fujimoto et al., 2014; Seemanova et al., 2014]. A review of these reports yielded five patients with NS and craniosynostosis (See Table I).

DISCUSSION

Craniosynostosis is not typically considered part of Noonan syndrome or other RASopathies. We report a child with Noonan syndrome due to a *KRAS* mutation (p.P34Q) and sagittal craniosynostosis and review all reported cases of RASopathies to identify similar patients with craniosynostosis. Including the present report, from a total of 62 cases of RASopathies with *KRAS* mutations, six patients had NS and craniosynostosis [Schubbert et al., 2006; Kratz et al., 2009; Lo et al., 2009; Brasil et al., 2012]. This suggests that individuals with Noonan syndrome associated with *KRAS* mutations have an increased risk for craniosynostosis with 1 of every 10 published cases of *KRAS* mutations having craniosynostosis; this is greater than 300 times the prevalence in the general population. Unlike other hereditary craniosynostosis syndromes, such as Muenke syndrome, which is largely confined to the coronal sutures, craniosynostosis associated with *KRAS* mutations affect multiple sutures including coronal, sagittal, metopic, and lamboid. There were no reports found describing patients with craniosynostosis and *KRAS*-associated CFC and/or phenotype suggesting CS.

The association of *KRAS* mutations and craniosynostosis is supported by the RAS/MAPK pathway's involvement in cranial development. The most common inherited craniosynostosis syndromes are caused by mutations in fibroblast growth factor receptors (FGFR) including Muenke, Pfeiffer, Apert, and Crouzon syndromes [for review see Cunningham et al., 2007]. FGFR proteins are receptor tyrosine kinases upstream of the RAS/MAPK signaling pathway [Kouhara et al., 1997; Lim et al., 1999]. Dysregulation of cranial development secondary to mutations in the

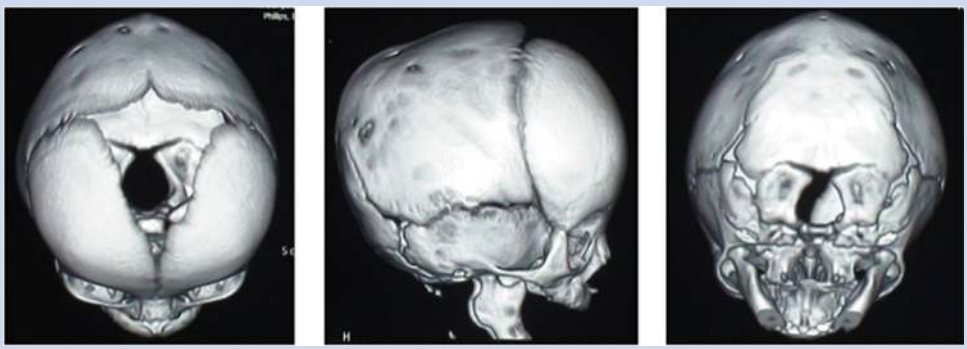

FIG. 3. Three-dimensional computed tomography reconstruction of skull showing sagittal suture fusion.

TABLE I. Clinical Findings of Patients With *KRAS* Mutations and Craniosynostosis

Patient	**1**	**2**	**3**	**4**	**5**	**6**
KRAS change	T58I	V14I	T58I	G60S	M72L	P34Q
Clinical diagnosis	NS	NS	NS	NS	NS	NS
Age	14mo	9y	3y	6y	6mo	2mo
Sex	F	M	M	M	M	F
Type of craniosynostosis	Sagittal	Sagittal	Sagittal and bicoronal	Left lambdoidal	Metopic	Sagittal
Short stature*	+	+	+	+	+	+
Dysmorphic facial features	+	+	+	+	+	+
Cardiac abnormality	ASD, VSD, VPS	PS	HRV, SH, OFO	DPV, PDA, SH	VPS	–
Developmental delay/Intellectual disability	+	+	+	+		
Skin findings	–	–	+		–	–
Characteristic hair	–	–	–	+	+	+
Chest wall deformities	+	+	–	+	–	–
Cryptorchidism	–	+			No	
Reference	Schubbert et al. [2006]	Lo et al. [2007]	Kratz et al. [2009]	Kratz et al. [2009]	Brasil et al. [2012]	This study
Other findings	JMML, webbed neck	Webbed neck, right sided sensorineural hearing loss, tinnitus, dizziness, vestibular aqueduct and cochlear dysplasia, delayed bone age	Double collecting system of the kidney, raised ICP, microcephaly, pterygium colli, cystic hygroma	GI reflux, Chiari 1 malformation, moderate sleep apnea, delayed bone age	Corneal nerves	Broad toes

ASD, atrial setpal defect; DPV, dysplastic pulmonary valve; HRV, hypertrophy of the right ventricle; ICP, increased cranial pressure; JMML, juvenile myelomonocytic leukemia; NS, Noonan syndrome; OFO, open foramen ovale; PS, pulmonary stenosis; PDA, patent ductus arteriosis; SH, septal hypertrophy; VSD, ventricle septal defect; VPS, valvular pulmonary stenosis.

*Short stature: < 10th centile.

FGFR genes is at least partially mediated by the genes in the RAS-MAPK pathway [Kim et al., 2003; Cunningham et al., 2007; Shukla et al., 2007]. In an animal model of Apert syndrome (*FGFR2*-related craniosynostosis syndrome), blocking activation of the RAS-MAPK pathway gene, *ERK1/2*, led to the prevention of craniosynostosis in mice [Shukla et al., 2007]. In another study, Twigg et al. [2013] showed that a reduced dosage of *ERF*, also a RAS-MAPK pathway gene, had an effect in the development of complex craniosynostosis.

The mutation found in our patient occurs in the highly conserved G domain (amino acids 1–165) of the KRAS protein, which is highly homologous with other Ras molecules [Gremer et al., 2011], with the first 85 amino acids involved in binding of guanosine diphosphate (GDP) and guanosine triphosphate (GTP) [Schubbert et al., 2007b]. Acting as a molecular switch, the KRAS protein is activated when bound to guanosine triphosphate (GTP) and inactivated when bound to guanosine diphosphate (GDP). NS-causing mutations in *KRAS* cause an abnormal upregulation of the protein function with impaired ability to switch between active and inactive states, subsequently leading to aberrant signal flow through the MAPK pathway [Schubbert et al., 2006, 2007a; Gremer et al., 2011]. The other reported *KRAS* mutations associated with craniosynostosis and all reported *KRAS* mutations in Noonan, Costello, and Cardio-facio-cutaneous syndromes are also located in the G domain (See Table I).

The mechanism for pathogenic mutations in components of the RAS-MAPK pathway leading to premature cranial synostosis is unknown. These mutations may not be exclusive to *KRAS*, but may include other RAS-MAPK genes responsible for NS phenotype. This is supported by the only other reported case of craniosynostosis in a child with a mutation in the RAS-MAPK pathway; Takenouchi et al. [2014] described a child with complex craniosynostosis (lambdoid and sagittal synostosis) and a mutation in *SHOC2* resulting in features consistent with Noonan syndrome.

The p.P34Q *KRAS* mutation in the present patient has been reported in another individual affected with NS without craniosynostosis [Zenker et al., 2007], demonstrating variable expressivity of the p.P34Q mutation. With the exception of patient two in Kratz et al. [2009], the previously reported mutations in patients with NS and craniosynostosis had also been reported in NS patients without craniosynostosis [Schubbert et al., 2006; Nava et al., 2007; Brasil et al., 2012]. These reports indicate that there are other genetic or environmental modifiers that influence the described abnormal cranial development. The variable expressivity of these mutations raises the possibility that aberrant mutations in the RAS-MAPK pathway may lead to covert abnormalities in cranial development where cranial sutures may radiographically appear patent, but have dysregulated growth in some parts of the sutures. Although no other reports aside from Takenouchi et al. [2014] link craniosynostosis with a *SHOC2* mutation, previous reports described individuals with *SHOC2*-associated NS as having abnormal head shape [Gripp et al., 2013].

In our and Takenouchi et al.'s [2014] patients, severe craniosynostosis masked the child's phenotype, making it difficult to diagnose NS, even though in retrospect, the child had Noonan-like features that initially escaped clinical recognition. This supports the need for future studies investigating the clinical prevalence of craniosynostosis due to mutations in other RAS-MAPK pathway genes, especially given that individuals with NS are at an increased risk of having congenital heart defects, and problems with coagulation and bleeding [Romano et al., 2010].

In conclusion, this case report and review of the literature shows that craniosynostosis is a significant finding in NS with *KRAS* mutations, supporting the role of the RAS/MAPK pathway in cranial suture closure.

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