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SHORT REPORT

Germline *PTPN11* and somatic *PIK3CA* variant in a boy with megalencephaly-capillary malformation syndrome (MCAP) - pure coincidence?

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Megalencephaly-capillary malformation (MCAP) syndrome is an overgrowth syndrome that is diagnosed by clinical criteria. Recently, somatic and germline variants in genes that are involved in the PI3K-AKT pathway (*AKT3*, *PIK3R2* and *PIK3CA*) have been described to be associated with MCAP and/or other related megalencephaly syndromes. We performed trio-exome sequencing in a 6-year-old boy and his healthy parents. Clinical features were macrocephaly, cutis marmorata, angiomata, asymmetric overgrowth, developmental delay, discrete midline facial nevus flammeus, toe syndactyly and postaxial polydactyly—thus, clearly an MCAP phenotype. Exome sequencing revealed a pathogenic *de novo* germline variant in the *PTPN11* gene (c.1529A>G; p.(Gln510Arg)), which has so far been associated with Noonan, as well as LEOPARD syndrome. Whole-exome sequencing (>100× coverage) did not reveal any alteration in the known megalencephaly genes. However, ultra-deep sequencing results from saliva (>1000× coverage) revealed a 22% mosaic variant in *PIK3CA* (c.2740G>A; p.(Gly914Arg)). To our knowledge, this report is the first description of a *PTPN11* germline variant in an MCAP patient. Data from experimental studies show a complex interaction of SHP2 (gene product of *PTPN11*) and the PI3K-AKT pathway. We hypothesize that certain *PTPN11* germline variants might drive toward additional second-hit alterations.
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INTRODUCTION

The megalencephaly-capillary malformation (MCAP) syndrome (OMIM #602501) is a clinically recognizable syndrome, consisting of megalencephaly and other typical features (see Table 1). It has first been described as macrocephaly-cutis marmorata (M-CM) teleangiectatica syndrome in 1997[1,2] and been renamed in 2007 to M-CM syndrome.[3] In order to reflect the very large brain size—rather than simply a large head—Mirzaa *et al*[4] proposed to re-rename the syndrome as MCAP syndrome, which is the current nomenclature until today.

Not surprisingly, there exist various diagnostic criteria for MCAP syndrome, differing in minor criteria depending on the respective authors.[5] To date, >140 cases have been reported in the literature.[6] Extensive work has been done to reveal the molecular causes and mechanisms of this, as well as the associated megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndrome.

Rivière *et al*[7] were the first to report on *de novo* germline and postzygotic variants in MCAP and MPPH syndromes in three key factors of the PI3K-AKT pathway: *AKT3*, *PIK3R2* and *PIK3CA*. In MCAP syndrome, only *PIK3CA* variants have been found (22 postzygotic and 2 germline variants).[6]

In this report, we describe the first patient with MCAP syndrome in whom exome sequencing revealed a germline *PTPN11* variant, whereas ultra-deep sequencing from saliva showed a 22% mosaic for a *PIK3CA* variant.

We discuss possible biological mechanisms that might be responsible for this intriguing observation.

CLINICAL REPORT

The index patient is a 6-year-old boy from consanguineous Turkish parents (cousins of second degree). Pregnancy was uneventful, but his mother has had two early miscarriages previously. Birth weight was 4230 g (98th centile), body length was 56 cm (98th centile) and head circumference was 36.5 cm (95th centile). APGAR scores were 10/10/10, and arterial umbilical pH was 7.34. Directly after birth, bilateral postaxial polydactyly and syndactyly II/III of the feet were detected.

Motor development, as well as speech development, were delayed. He learned to walk at 22 months.

Examination at the age of 6 years and 3 months: Friendly and cooperative boy. Striking features were cutis marmorata, macrocephaly (head circumference 56.5 cm, +2.9 SD>98th centile), frontal bossing, hemangiomata (nasal root, tongue and lower lip), discrete facial nevus flammeus and hemihypertrophy of the left body part. Postaxial hexadactyly had been operated; syndactyly II/III had only been operated on the right side. According to the clinical criteria,[5] the diagnosis MCAP syndrome could be established (see Table 1; some clinical features are shown in Figure 1).

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Table 1 Synopsis of the MCAP an Noonan phenotypes

MCAP phenotype	*Noonan phenotype*
Segmental overgrowth	Facial dysmorphism
(hemi-)*megalencephaly*[a]	*Epicanthal folds*
Cerebellar tonsillar ectopia or Chiari malformation	Ptosis
Abnormal thick (mega-) *corpus callosum*	*Down-slanting palpebral fissures*
Congenital somatic overgrowth	Triangular facies *low set and/or posteriorly rotated ears*
Somatic or cranial *asymmetry*	
Vascular disorders	Ophthalmologic
Cutaneous capillary malformations[a] *(e.g., midline facial nevus flammeus, hemangiomata, cutis marmorata)*	Strabismus, myopia
	Hearing loss
	Dental/oral features
Digital anomalies	Cardiovascular features
Syndactyly[a]	Congenital heart defects (various)
Postaxial polydactyly	
Cortical brain malformations	Webbed and *short neck with low posterior hair line*
Polymicrogyria	
	Feeding difficulties
Connective tissue dysplasia	
Skin hyperelasticity[a]	*Developmental delay*
Joint hypermobility[a]	
Thick, soft subcutaneous tissue[a]	Skeletal features
Others[b]	Cryptorchism
Hypotonia	
Developmental delay	Lymphatic features
Fronal bossing	Lymphedema, lymphangiectasia
Seizures	
	Hematological features
	Bleeding diathesis
	Leukemia

Abbreviation: MCAP, megalencephaly-capillary malformation.
[a]MCAP: these items are considered 'major criteria', whereas the other items are considered 'supportive criteria'.
[b]The other findings are considered to be 'secondary'. Italics: our patient complied with these criteria.
Adapted from Mirzaa *et al*[5] and Tartaglia *et al*.[11] Although some symptoms of the Noonan phenotype are present in our index patient, the MCAP phenotype is much more distinctive.

METHODS AND RESULTS

After array-CGH analysis and candidate gene sequencing of the megalencephaly genes *AKT3*, *PIK3R2* and *PIK3CA* of leukocyte DNA by conventional methods (Sanger sequencing) had returned normal results, we performed trio-exome sequencing of the boy and his parents. The diagnostic pipeline was as previously reported.[8]

Exome sequencing of leukocyte DNA revealed a *de novo* heterozygous variant in the *PTPN11* gene (c.1529A>G; p.(Gln510Arg); according to LRG_614, NG_007459.1, NM_002834.3 and ENST00000351677). Both parents did not carry this variant. Paternity was confirmed. This finding was confirmed by Sanger sequencing from buccal swab DNA, therefore suggesting a germline *PTPN11* variant. As that variant has been described to be associated with Noonan[9] and LEOPARD[10] syndrome and thus did not seem to explain the patient's complete phenotype, special attention was payed to the megalencephaly genes *PIK3CA*, *PIK3R2* and *AKT*. In DNA from blood, no variant could be detected (coverage: >150-fold). Deep sequencing results of 551 tumor-associated genes from saliva (CeGaT somatic tumor panel TUM01, version 2; http://cegat.de/Tumor-panel-%28somatic%29_l=1_202.html, including *PIK3CA*, *PIK3R2* and *AKT*; coverage: >1000-fold) revealed a known disease-causing variant in *PIK3CA* (c.2740G>A; p.(Gly914Arg); according to LRG_310, NG_012113.2, NM_006218.2 and ENST00000263967) in 172 out of 1572 reads, thus indicating that 22% of the cells carry the variant in a heterozygous state (somatic mosaic).

The patient phenotype and the detected variants have been submitted to the Leiden Open Variation Database (LOVD 3.0; http://databases.lovd.nl/shared/individuals/00016593).

DISCUSSION

Variants in the RAS-MAPK pathway are known as the cause of several entities of the neuro-cardio-facio-cutaneous syndrome spectrum, which share many clinical features but, depending on the location of the variant in the pathway, display distinguishable phenotypes. The most common phenotype is Noonan syndrome (the prevalence is estimated to be 1:1000–1:2500), but there are other distinct phenotypes such as LEOPARD (lentigines, electrocardiographic abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation, deafness) syndrome, cardio-facio-cutaneous syndrome, Costello syndrome, neurofibromatosis type 1 and Legius syndrome.

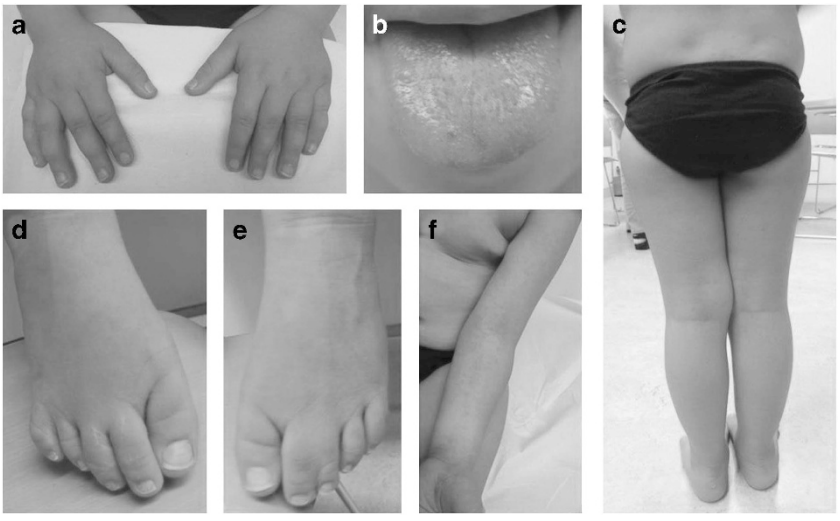

Figure 1 (**a**–**f**) Clinical features of the index patient. (**a**) hands; (**b**) hemangioma located on the tongue; (**c**) hemihypertrophy of the left leg; (**d**) right foot (syndactyly has been operated, as well as postaxial polydactyly); (**e**) left foot (remaining syndactyly II/III); (**f**) cutis marmorata of the left arm.

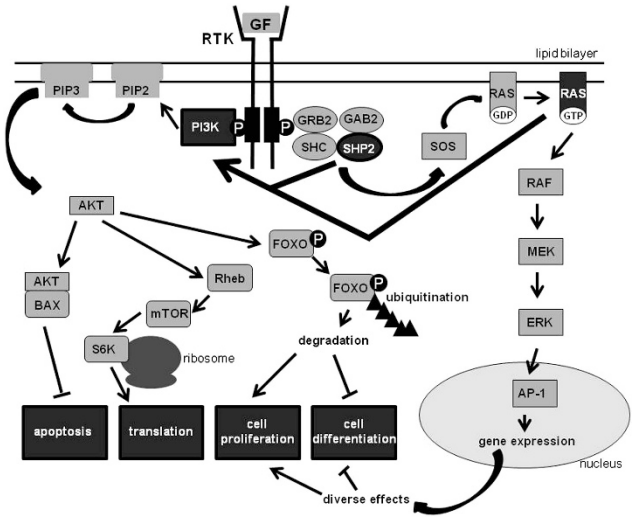

Figure 2 Simplified synopsis on the PI3K-AKT and RAS-MAPK signaling pathways and their interactions. The signaling cascades start at a receptor tyrosine kinase (e.g., a growth hormone receptor). Shown on the left is the PI3K-AKT pathway, on the right the RAS-MAPK pathway. (Some) interactions of the pathways are highlighted by thick arrows: activated RAS is known to interact with PI3K, activating its downstream signaling. Furthermore, SHP2 (the protein encoded by *PTPN11*) also interacts with the PI3K pathway. The effects are shown in the red boxes: inhibition of apoptosis, start of translation, induction of cell proliferation and inhibition of cell differentiation. All of these are possible underlying mechanisms not only of MCAP, but also of tumorigenesis. GF, growth factor; RTK, receptor tyrosine kinase; PI3K, phosphatidyl-inositol-3-kinase; PIP2, phosphatidyl-inositol-bisphosphate; PIP3, phosphatidyl-inositol-trisphosphate; AKT, PKB (protein kinase b); BAX, Bcl-2-like protein 4; Rheb, Ras homolog enriched in brain; mTOR, mammalian target of rapamycin; S6K, S6 kinase; FOXO, forkhead box protein O; GRP2, growth factor receptor-bound protein 2; GAB2, GRB2-associated protein 2; SHC, Src 2 homology domain containing; SHP2, gene product of *PTPN11*; SOS, son of sevenless; RAS, rat sarcoma protein; RAF, RAS-associated factor; MEK, mitogene-activated protein kinase kinase, ERK, extracellular signal-regulated kinase; AP-1, activating protein 1. The full colour version of this figure is available at *European Journal of Human Genetics* online.

As the variants all lead to functionally altered proteins in the RAS-MAPK pathway, this group is also referred to as 'rasopathies'.[11,12]

Recently, variants in the PI3K-AKT pathway have been described to be associated with megalencephaly and overgrowth syndromes, such as MCAP and MPPH,[5–7] but also CLOVES (congenital lipomatous asymmetric overgrowth, epidermal naevi, skeletal and spinal anomalies) syndrome,[13,14] as well as hemimegalencephaly[15] and isolated macrodactyly.[16]

Although both the RAS-MAPK pathway and the PI3K-AKT pathway are very complex and still only partly understood, interactions between these two signaling pathways are well known: most importantly but not exclusively, activated GTP-bound RAS not only induces the MAP-kinase cascade through association with RAF, but can also bind to PI3K to increase the generation of PIP3 out of PIP2, thereby activating several downstream effectors such as mTOR, BAX or FOXO—each directing to distinct cellular results leading to inhibition of apoptosis, initiation of translation, cell proliferation and loss of cell differentiation (see Figure 2).

Apart from implications in neurodevelopmental disorders, both pathways are extensively described in tumorigenesis[17] and many of the above mentioned syndromes are associated with a higher risk for the development of benign and/or malignant tumors.

For example, in MCAP syndrome, many benign vascular tumors (described as hemangiomas, angiomata and angiomyolipomas),[5] but also two meningeomas,[1,18] as well as a few malignant tumors have been reported (two children with Wilms tumor[19,20] and leukemia in an 18-year-old[1]).

In Noonan patients, juvenile myelocytic leukemia (JMML) occurs more often than in the general population.[11] As biologically suspected, disruption of the RAS-MAPK pathway lowers the transformation rate to tumor cells *in vitro*.[21] Interestingly, also intervention on the PI3K-AKT pathway has been shown to have positive effects: AKT phosphorylation is known to be elevated in PTPN11-induced JMML, and also mTOR inhibitors have been shown to be effective in *in vitro* studies.[21–24] This intriguing observation has been confirmed by animal studies. In line with the *in vitro* data, there is evidence that hypertrophic cardiomyopathy caused by specific *Ptpn11* variants in cell lines and mouse models of LEOPARD syndrome can be successfully treated by mTOR inhibition (rather than MEK inhibition).[25–28]

Thus, it can be concluded that alteration of both the MAP-kinase cascade and the PI3K-AKT effector pathway are involved in the pathophysiology of rasopathies. Certain organ manifestations may be mediated not only by one of these two pathways, but by a specific interaction of the two. It should be noted that this interaction might be tissue specific and time dependent.

This hypothesis is further supported by the following observations: during the last years, oncogenomics revealed that *PIK3CA* variants frequently coexist with *RAS* and *BRAF* variants in patients with advanced cancer.[29] A very recent publication even showed that in 4 out of 50 advanced gastric cancers, *PIK3CA* and *PTPN11* were both mutated, leading to activation of the AKT pathway.[30]

This seems to be more than just coincidence. It is known that in most cancers, there is not only one gene that is mutated, but a distinct set of genes. Not all of these variants seem to be relevant: most of the alterations are described to be 'passenger mutations', indicating their rather passive role in tumorigenesis. However, some are known to be 'driver mutations' and are essential for tumor progression.

MCAP being an overgrowth syndrome is pathogenically closely related to tumor disorders, as the same pathways are affected. Therefore, it is possible and biologically plausible that in our patient, the *PTPN11* variant is not just coincidental, but preceding (or even causing) the *PIK3CA* variant.

Further studies, for example, systematic screening of MCAP patients for alterations in the RAS-MAPK pathway, are needed to confirm this hypothesis. Especially in the context of tumor risk, the knowledge of a second alteration in another pathway might not just be academically interesting, but very valuable for planning individual risk assessment strategies.

Our 'second hit' hypothesis is further supported by the first patient reported in the literature carrying the same *PTPN11* variant (c.1529A>G; p.(Gln510Arg)).[9] Apart from the *PTPN11* variant, the female index patient in this publication also showed an *NF1* variant. She exhibited symptoms of both phenotypes, whereas her father who only had the *PTPN11* variant was described to have a Noonan phenotype.[9]

In conclusion, there is a lot of evidence that activation of both the RAS-MAPK pathway, as well as the PI3K-AKT pathway has a major role in the pathogenesis of rasopathies. In tumors, both of these effector pathways seem to be associated not only in a functional way, but on DNA level, as variants in effectors of both pathways co-occur frequently in some advanced tumors. The same mechanism might be responsible for the phenotype presented in this report.

Further patient and animal studies are needed to address the link between overgrowth syndromes and rasopathies. Clinicians are encouraged to search for somatic variants in patients with unusual clinical features by using next-generation sequencing approaches. It should be noticed that somatic variants might not be detectable from blood, so analysis of a second tissue such as saliva or skin should be considered.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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