European Journal of Medical Genetics 55 (2012) 191–195

Contents lists available at SciVerse ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Original article

Phenotypic variability in a family with capillary malformations caused by a mutation in the *RASA1* gene

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ARTICLE INFO

Article history:
Received 8 November 2011
Accepted 21 January 2012
Available online 28 January 2012

Keywords:
RASA1
RasGAP
CM-AVM
Capillary malformation
Phenotype
Etiology

ABSTRACT

Hereditary capillary malformations are known to be caused by mutations in the *RASA1* gene. The associated phenotype is still subject of debate. The purpose of this study was to conduct a *RASA1* mutation analysis in the family that led to the initial discovery of the 5q locus, and to delineate the associated phenotype. A novel truncating mutation was identified in all clinically affected individuals and in none of the unaffected members. The associated phenotype was widely variable; all individuals had multifocal CM with at least one area of high flow. Various additional features were observed, some previously reported and others novel, including limb overgrowth, varicosities, possible lymphatic malformations, localized hyperhidrosis and exercise induced redness. The cause of this wide intramutational phenotypic variability remains to be elucidated.

1. Introduction

Capillary malformations (CMs, or port-wine stains; OMIM 163000) are the most common vascular malformations, occurring in 0.3% of live births [1].

Although CMs are usually sporadic, familial occurrence of multiple CMs do occur and was first described by Shelley and Livinghood in 1949 [2]. Since then a limited number of similar families have been reported [3–6], and segregation patterns suggested an autosomal dominant mode of inheritance with variable expression.

In 2002, our group identified a locus for hereditary CM at 5q13-q22 [7], and subsequently Eerola and co-workers identified *RASA1*, located at 5q13.3, as a gene causing atypical CM and high flow arteriovenous malformations (CM-AVM; OMIM 608354) in 6 families [8]. Several studies [9–12] found *RASA1* mutations to have a high penetrance (>90%) and a widely variable associated phenotype. Here we report on the results of a *RASA1* mutation analysis in the original family that allowed linkage to the 5q locus, and provide an update of the associated phenotype.

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1769-7212/$ – see front matter © 2012 Published by Elsevier Masson SAS.
doi:10.1016/j.ejmg.2012.01.009

2. Materials and methods

2.1. Patients

All members of the family reported by Breugem et al. [7] (Fig. 1) were contacted, and all provided consent to further mutation analysis. The patients filled out a detailed questionnaire and were clinically examined by two investigators (RSDW; CEUO) for presence and characteristics of CM, abnormalities in limb length and circumference, the presence of varicose veins and signs of lymphatic malformations. In addition, CMs were examined for areas of high flow by hand held Doppler ultrasound. Standardized digital photographs were taken of all lesions.

2.2. Molecular genetics

DNA was extracted from peripheral lymphocytes using standard methods. Primers were developed to amplify all coding exons of *RASA1* (gene reference sequence NM_002890.1; primer sequences available on request). Polymerase chain reaction (PCR) fragments were bidirectionally sequenced using the Bigdye kit v1.1 (Applied Biosystems). Reactions were run on an ABI3700 genetic analyzer

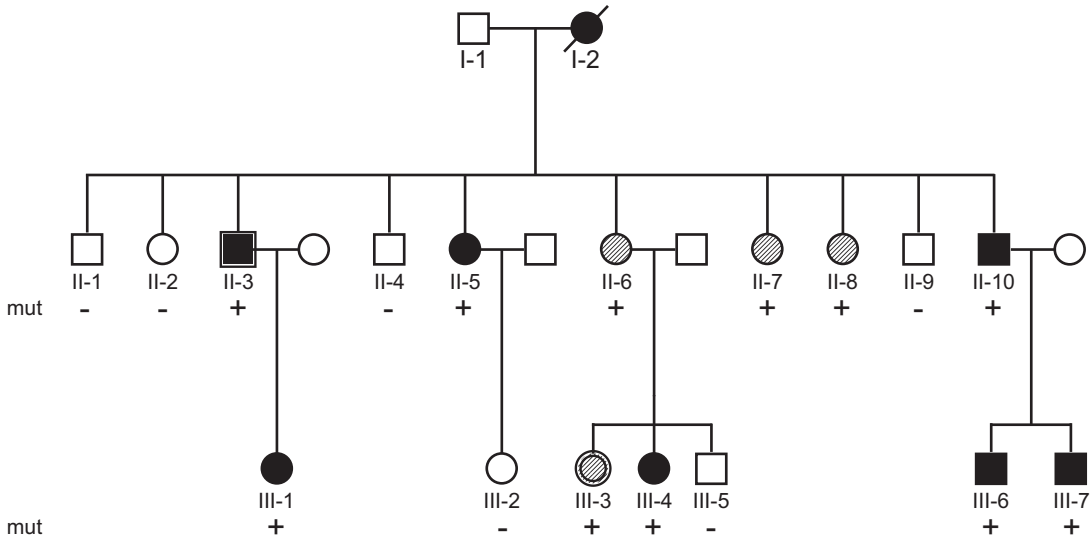

Fig. 1. Pedigree. The presence of the *RASA1* mutation is indicated by a '+'. Clinically affected individuals are filled by a solid color (CM and AVM) or striped pattern (CM, AVM and varicose veins). A double wall indicates limb hypertrophy.

(Applied Biosystems) and sequences were analyzed using codoncode aligner (Codoncode Corporation).

3. Results

3.1. Molecular genetics

Direct sequencing of the coding exons of *RASA1* showed a novel heterozygous complex mutation c.734_737delinsAAA in exon 3 in all affected individuals and in none of the unaffected members. This insertion-deletion results in a frameshift starting at codon Arg245 and a premature stop codon after 7 altered aminoacids (p.Arg245fsX8) (Fig. 2).

3.2. Patients

None of the patients without *RASA1* mutation had a vascular or other relevant physical finding, although patient III-2 had a hemangioma. All patients with the *RASA1* mutation had at least one CM (mean number 4; range 2–10) of varying size (mean diameter 11.7 cm; range 1–46 cm). Appearance varied from round-to-oval, red-pink lesions to irregularly-shaped purple lesions. Some lesions were warm to touch, but none had a palpable thrill. Doppler ultrasound showed at least one area of high flow within the CM in all patients (mean number 1.9; range 1–4) suggestive of an arteriovenous anomaly. There was no clear association between the size of a CM and the presence of an area of high flow. Four lesions had a white halo suggestive of vascular steal [9]. These findings are summarized in Table 1.

In addition to the signs mentioned in Table 1, various unusual characteristics were observed. Two patients had associated limb overgrowth: patient II-3 had a lesion covering approximately half of the left leg with an increased circumference (10 cm) (Fig. 3) He was known since childhood with unilateral edema of the lower leg requiring a pressure stocking, and earlier histological analysis had suggested this to be a lymphatic malformation. We have been unable to confirm this more recently. Patient III-3 had a CM covering the left upper leg and a mildly (2.5 cm) increased circumference of the entire leg. Four patients (II-6, II-7. II-8 and III-3) had early-onset varicose veins (Fig. 4), of which two (II-7 and II-8) reported a burning redness of the lower legs induced by a combination of warm weather and exercise that subsided spontaneously over several days (Fig. 5). In patient II-7, an area of localized hyperhidrosis was identified in a CM with high flow on her left forearm Finally, in two patients (III-1 [with mutation] and III-2 [without mutation]), a 3 cm haemagioma had been present on the head during infancy and childhood and a salmon patch had been present on the forehead of III-6 as an infant.

4. Discussion

RASA1 (RAS p21 protein activator 1; OMIM 139150) encodes the protein p120 RasGAP. It is one of 14 human Ras GTPase activating proteins [13], that primarily function as a negative regulator of proto-oncogenic Ras [14]. The non-catalytic domain of these proteins differs in composition and mediates additional effects. In p120 RasGAP, it regulates GAP activity [15-18] and also serves as an effector of Ras [19] by binding to growth factor receptors [20] and cytoplasmatic proteins [21,22].

Therefore RASA1 is not only a regulator of cellular differentiation and proliferation, but also has functions in cytoskeletal reorganization [23], cell migration [24] and survival [16,25]. *RASA1* knockout models have a defective angiogenesis [26], in which the severity of vascular defects correlates with the level of residual RasGAP expression, and mosaic embryos develop localized defects [27]. Recently, microRNA-132 was shown to cause suppression of RasGAP function and increased Ras activity, leading to pathological neovascularisation. Interestingly, this effect could be reversed by anti-microRNA-132 [28].

Activating mutations in the Ras proto-oncogenes are common in many tumors [29]; it could therefore be hypothesized that defective RasGAP would leave Ras in its active state causing uncontrolled cellular proliferation. Indeed, modulation of p120 RasGAP levels has been observed in various malignancies such as chronic myelogenous leukemia [30], astrocytoma [31], trophoblastic tumors [32], prostate cancer [33] and basal cell carcinoma [34]. Mutations of the GAP domain however have not been demonstrated; an analysis of lung cancer cell lines [35] showed no results and mutations identified in basal cell carcinoma were located in the non-catalytic domain [36], indicating the causative mechanism might be independent of Ras.

Mutations in *NF1*, a RasGAP homolog, can cause neurofibromatosis type 1 [37]. Indeed, a small subset of neurofibromatosis 1 patients also exhibits vascular abnormalities, such as aneurysms, stenoses and ateriovenous malformations [38], and there is