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Nevus Sebaceus Is a Mosaic RASopathy

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The recent discovery that nevus sebaceus is a mosaic RASopathy represents a major breakthrough in research on epidermal nevi. In this issue, both Levinsohn *et al.* and Sun *et al.* confirm this advancement with results obtained through whole-exome sequencing. Further molecular studies will almost certainly show that sebaceous and keratinocytic nevi are different disorders, although there is some clinical overlap.

Journal of Investigative Dermatology (2013) **133,** 597–600. doi:10.1038/jid.2012.447

Nevus sebaceus is common among epidermal nevi. When involving the head, lesions consist of linear or patchy, skin-colored or yellowish plaques with hairless, orange peel–like surfaces (Figure 1). In the era of molecular research, it took a rather long time to elucidate the genetic basis of this disorder. However, the question has been answered with the identification of postzygotic *HRAS* and *KRAS* mutations as the cause of both isolated nevus sebaceus and the Schimmelpenning syndrome (Groesser *et al.*, 2012). In the present issue, two American groups present interesting findings that fully confirm the results reported by the European authors.

In this commentary, we shall consider how this major breakthrough in research on epidermal nevi was accomplished and the new questions that this advancement poses.

A discovery achieved by the candidate gene method

One year ago, the group of Christian Hafner from Regensburg (Germany) reported, in collaboration with Spanish scientists, that they had found an activating *HRAS* mutation in a patient who had a systematized keratinocytic nevus associated with early development of urothelial cancer (Hafner *et al.*, 2011). Subsequently, screening of keratinocytic nevi revealed activating *RAS* mutations in 39% of cases, with the *HRAS* p.G13R substitution being a hotspot mutation (Hafner *et al.*, 2012). As a further step, the authors then decided to look, using a candidate gene approach, for the presence of *RAS* mutations in nevus sebaceus.

By using Sanger sequencing and a *RAS* snapshot multiplex assay, they found *HRAS* or *KRAS* mutations in 63/65 sebaceous nevi (97%). In all, 95% of lesions harbored an *HRAS* mutation, with *HRAS* c.37G>C representing a hotspot mutation encountered in 91% of cases. *KRAS* mutations were found in 5% of sebaceous nevi. All of these mutations were present in a heterozygous state.

In a patient with Schimmelpenning syndrome, the authors found the *HRAS* c.37G>C mutation exclusively in lesional skin. In another patient with this syndrome, a *KRAS* mutation was noted to be present in lesional tissue only. When analyzing secondary tumors that had developed in sebaceous nevi, they detected the same *HRAS* mutations that were present in the underlying nevi. The authors concluded that both nevus sebaceus and Schimmelpenning syndrome could be categorized as mosaic RASopathies.

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Confirming the etiology of nevus sebaceus by whole-genome sequencing

Levinsohn *et al.*, 2013 (this issue) present a complementary study that provided similar results. In contrast to the candidate gene approach used by Groesser *et al.*, the authors performed whole-exome sequencing of DNA obtained from blood and lesional tissues. Again, *HRAS* c.37G>C was found to be a hotspot mutation. Furthermore, the *KRAS* mutations c.35G>A and c.35G>T were encountered in two cases. They were identical to the two *KRAS* alleles described by Groesser *et al.* Notably, the authors confirmed the absence of any loss of heterozygosity.

In several specimens obtained from tumors that arose on sebaceous nevi, the authors confirmed the presence of *HRAS* mutations.

Confirming the etiology of Schimmelpenning syndrome

In another paper, Sun *et al.*, 2013 (this issue) present molecular data obtained in a 38-year-old woman with multiple features of Schimmelpenning syndrome, including severe neurological defects and pronounced scoliosis. In her third decade, she experienced a cerebral stroke. Samples obtained from her sebaceous nevus were analyzed by exome sequencing. Again, the authors encountered the *HRAS* hotspot mutation c.37G>C. Moreover, they found this hotspot mutation in 24/31 archived tissue samples obtained from independent sebaceous nevi. Other *HRAS* or *KRAS* mutations were documented in three of these specimens. In total, 89% of samples showed *HRAS* or *KRAS* mutations.

Comparison with other mosaic RASopathies

The ''RASopathies'' represent a group of autosomal dominant syndromes characterized by dysregulation of the RAS/MAPK pathway (Tidyman and Rauen, 2009). Recently, the term ''mosaic RASopathies'' was proposed for disorders resulting from activation of this pathway by mosaic mutations (Hafner *et al.*, 2012). A list of the reported mosaic RASopathies is presented in Table 1. Nevus sebaceus belongs to a

Clinical Implications

- Nevus sebaceus, including Schimmelpenning syndrome, is caused by postzygotic *HRAS* or *KRAS* mutations.
- The disorder can be added to a list of mosaic RASopathies that includes a specific group of keratinocytic nevi and mosaic cases of Costello syndrome, neurofibromatosis 1, Legius syndrome, rhodoid nevus syndrome, and LEOPARD syndrome.
- Familial nevus sebaceus can no longer be explained by the concept of paradominant inheritance, because the underlying *RAS* mutations have been shown to be present in a heterozygous state.

group of disorders caused by lethal mutations that survive through mosaicism (Happle, 1987). A large part of keratinocytic nevi are similarly caused by *RAS* mutations that, when present as a germ-line mutation, would be lethal for an embryo early in development.

A mosaic RASopathy reflecting a nonlethal mutation is exemplified by segmental forms of Costello syndrome. This autosomal dominant multisystem birth defect is characterized by various cutaneous lesions such as generalized hyperpigmentation, loose skin (especially redundant on the hands), periorificial papillomas, velvety appearance of palms and soles that show relatively deep creases, and acanthosis nigricans–like hyperkeratosis. The patients' hair is sparse, brittle, and curly. In a 15-year-old girl with features of Costello syndrome, Gripp *et al.* (2006) found segmental areas of hyperpigmentation on her trunk and right arm, streaky hyperpigmention on the dorsal aspect of her left foot, and segmental hyperkeratosis involving her left sole. The authors found an *HRAS* mutation p.G12S in sampled buccal cells, whereas her blood leukocytes showed the wild type only. A similar case of segmental Costello syndrome caused by a mosaic *HRAS* mutation was reported by Sol-Church *et al.* (2009).

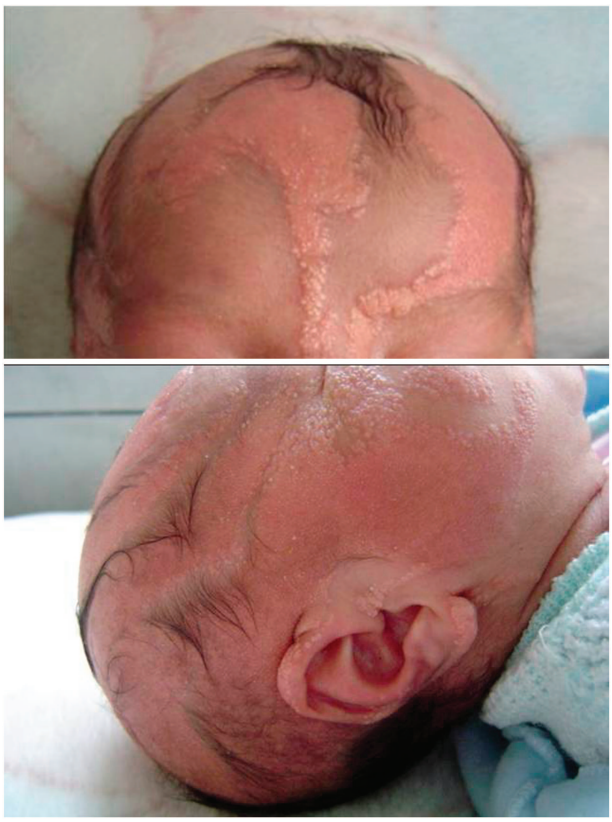

Figure 1. Systematized sebaceous nevus involving the scalp and the face (courtesy of Dr Mónica Zambrano, Quito, Ecuador).

In some other autosomal dominant RASopathies, a type 2 segmental involvement, being superimposed on the ordinary nonsegmental phenotype (Happle, 1997), can be taken as proven at the molecular level. In the first report on a phenotype that later was named Legius syndrome, Brems *et al.* (2007) included a photograph suggesting a type 2 segmental manifestation in the form of an extensive, flag-like café-au-lait hyperpigmentation being sharply demarcated in the midline. This large unilateral patch may be best explained by an early event of allelic loss (Fölster-Holst *et al.*, 2012).

Writzl *et al.* (2007) described LEOPARD syndrome in a man who fathered a son whose skin was similarly pigmented except for the left side of his thorax, back, and left arm, which were completely devoid of lentigines. Among several possibilities, the authors discussed revertant mosaicism, which appears to be the most likely explanation.

Are sebaceous and keratinocytic nevi variants of the same disorder?

When a systematized sebaceous nevus involves several parts of the body, the components affecting the trunk and limbs usually do not show hyperplasia of sebaceous glands, which is why these portions of the nevus may be indistinguishable from a keratinocytic nevus (Happle, 1995). This has led some authors to the erroneous belief that sebaceous and keratinocytic nevi are variants of the same disorder (Solomon and Esterly, 1975; Waltz *et al.*, 1999; Moss and Shadihullah, 2010). Molecular studies, however, have shown that this view cannot be sustained because a quite different spectrum of *HRAS* mutations is found in keratinocytic nevi (Hafner *et al.*, 2012). When such nevi involve the head, they do not show hyperplasia of sebaceous glands.

Nevus sebaceus can no longer be regarded as a paradominant trait

Familial aggregation of nevus sebaceus has been reported relatively frequently.

Table 1. Synopsis of mosaic RASopathies as presently known

Disorder	MIM number	Gene	Type of mosaicism	References
Nevus sebaceus	163200	*HRAS* *KRAS*	Lethal mutation surviving by mosaicism	Groesser *et al.*, 2012; Levinsohn *et al.*, 2012; Sun *et al.*, 2012
Keratinocytic nevus	162900[a]	*HRAS*	Lethal mutation surviving by mosaicism	Hafner *et al.*, 2012
Costello syndrome	218040	*HRAS*	Type 1 segmental manifestation of an autosomal dominant trait	Gripp *et al.*, 2006; Sol-Church *et al.*, 2009
Neurofibromatosis 1	162200	*NF1*	Type 1 segmental manifestation of an autosomal dominant trait Type 2 segmental manifestation of an autosomal dominant trait	Tinschert *et al.*, 2000 Happle, 2001; Steinmann *et al.*,2009
Legius syndrome	611431	*SPRED1*	Type 2 segmental manifestation of an autosomal dominant trait	This commentary
Rhodoid nevus syndrome (capillary malformation–arteriovenous malformation)	608354	*RASA1*	Type 2 segmental manifestation of an autosomal dominant trait	Eerola *et al.*, 2003; Happle, 2010
LEOPARD syndrome	151100	*PTPN11*	Revertant mosaicism (?)	Writzl *et al.*, 2007

[a]In addition to keratinocytic nevus, this entry includes several quite different disorders.

As random coincidence appeared to be unlikely, we suggested the concept of paradominant transmission (Happle and König, 1999). Accordingly, a heterozygous individual would be phenotypically healthy. The nevus would only develop when early postzygotic recombination gave rise to loss of the corresponding wild-type allele. As nevus sebaceus has now been shown to be caused by a heterozygous state of postzygotic *RAS* mutations, the concept of paradominance of this trait must be regarded as incorrect. Perhaps the familial cases may be explained by an unstable premutation, but readers should understand that at this point I prefer not to propose additional hypotheses.

Woolly hair nevus: another mosaic RASopathy?

Woolly hair nevus is characterized by linear areas of curly or woolly hair noted within straight scalp hair. The surface of the involved skin may appear normal, or the lesion may belong to a systematized keratinocytic nevus and thus may be hyperkeratotic. As curly hair is a feature of various RASopathies such as Costello syndrome, Noonan syndrome, and cardiofaciocutaneous syndrome, woolly hair nevus should be examined for *HRAS* mutations. In this context, however, it is tempting to speculate that keratinocytic nevi caused by *HRAS* mutations may give rise to curly or woolly scalp hair, whereas other keratinocytic nevi may not.

Conclusion

The knowledge that nevus sebaceus, including Schimmelpenning syndrome, is caused by *HRAS* and *KRAS* mutations leads to several important questions. Why is nevus sebaceus characterized by dichotomous clinical and histopathological changes, depending on the involved area of the body? How can we explain familial nevus sebaceus, as the theory of paradominant inheritance is no longer valid for this trait? Do other birthmarks, such as woolly hair nevus, represent additional candidates to be tested for the presence of *RAS* mutations? Addressing these problems will yield further insight into the relationship between dysregulation of the RAS/MAPK pathway and skin disorders.

CONFLICT OF INTEREST

The author states no conflict of interest.

REFERENCES

Brems H, Chmara M, Sahbatou M *et al.* (2007) Germline loss-of-function mutations in *SPRED1* cause a neurofibromatosis 1-like phenotype. *Nat Genet* 39:1120–6

Eerola I, Boon LM, Mulliken JB *et al.* (2003) Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by *RASA1* mutations. *Am J Hum Genet* 73:1240–9

Fölster-Holst R, Nellen RG, Jensen JM *et al.* (2012) Molecular genetic support for the rule of dichotomy in type 2 segmental Darier disease. *Br J Dermatol* 166:464–6

Gripp KW, Stabley DL, Nicholson L *et al.* (2006) Somatic mosaicism for an *HRAS* mutation causes Costello syndrome. *Am J Med Genet* 140A:2163–9

Groesser L, Herschberger E, Ruetten A *et al.* (2012) Postzygotic *HRAS* and *KRAS* mutations cause nevus sebaceus and Schimmelpenning syndrome. *Nat Genet* 44:783–7

Hafner C, Toll A, Gantner S *et al.* (2012) Keratinocytic epidermal nevi are associated with mosaic *RAS* mutations. *J Med Genet* 49: 249–53

Hafner C, Toll A, Real FX (2011) *HRAS* mutation mosaicism causing urothelial cancer and epidermal nevus. *N Engl J Med* 365: 1940–2

Happle R (1987) Lethal genes surviving by mosaicism: a possible explanation for sporadic birth defects involving the skin. *J Am Acad Dermatol* 16:899–906

Happle R (1995) Epidermal nevus syndromes. *Semin Dermatol* 14:111–21

Happle R (1997) A rule concerning the segmental manifestation of autosomal dominant skin disorders: review of clinical examples providing evidence for dichotomous types of severity. *Arch Dermatol* 133: 1505–9

Happle R (2001) Large plexiform neurofibromas may be explained as a type 2 segmental manifestation of neurofibromatosis 1. *Am J Med Genet* 98:363–4

Happle R (2010) The rhodoid nevus: a proposed term for a so far unnamed capillary malformation. *Dermatology* 221:317–9

Happle R, König A (1999) Familial naevus sebaceus may be explained by paradominant transmission. *Br J Dermatol* 141:377

Levinsohn JL, Tian LC, Boyden LM *et al.* (2013) Whole exome sequencing reveals somatic mutations in *HRAS* and *KRAS* which cause nevus sebaceus. *J Invest Dermatol* 133: 827–30

Moss C, Shadihullah H (2010) Naevi and other developmental defects. In: Burns T, Breathnach S, Cox N, Griffiths C (eds) *Rook's Textbook of Dermatology*. 8th edn. Wiley-Blackwell: Chichester, West Sussex, UK pp. 18.1–107

Sol-Church K, Stabley DL, Demmer LA *et al.* (2009) Male-to-male transmission of Costello syndrome: G12S *HRAS* germline mutation inherited from a father with somatic mosaicism. *Am J Med Genet* 149A:315–21

Solomon LM, Esterly NB (1975) Epidermal and other congenital organoid nevi. *Curr Probl Pediatr* 6:1–56

Steinmann K, Kluwe L, Friedrich RE *et al.* (2009) Mechanisms of loss of heterozygosity in neurofibromatosis type 1-associated plexiform neurofibromas. *J Invest Dermatol* 129:615–21

Sun BK, Saggini A, Sarin KY *et al.* (2013) Mosaic activating *RAS* mutations in nevus sebaceus and nevus sebaceus syndrome. *J Invest Dermatol* 133:824–7

Tidyman WE, Rauen KA (2009) The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. *Curr Opin Genet Dev* 19:230–6

Tinschert S, Naumann I, Stegmann E *et al.* (2000) Segmental neurofibromatosis is caused by somatic mutation of the neurofibromatosis type 1 (NF1) gene. *Eur J Hum Genet* 8:455–9

Waltz KM, Helm KF, Billingsley EM (1999) The spectrum of epidermal nevi: a case of verrucous epidermal nevus contiguous with nevus sebaceus. *Pediatr Dermatol* 16:211–3

Writzl K, Hoovers J, Sistermans EA *et al.* (2007) LEOPARD syndrome with partly normal skin and sex chromosome mosaicism. *Am J Med Genet* 143A:2612–5