COMMENT

Patients carrying Arg1809 substitution with no choroidal abnormalities: a further proof of a "Quasi-Incomplete" NF1 phenotype

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Neurofibromatosis type 1 (NF1; MIM 162200) is a genodermatosis due to heterozygous mutations in *NF1* (MIM 613113) that explains why it is also classified among RASopathies. The diagnosis is based on recently revised criteria that include minimal clinical and genetic criteria for diagnosing and differentiating NF1 and Legius syndrome (LGSS; MIM 611431) [1]. These include typical café-au-lait macules (CALMs, at least six and with an age specific diameter), skinfold freckles, Lisch nodules (LNs) and choroidal abnormalities (CAs), neurofibromas, skeletal dysplasia and optic glioma. One further criterion is represented by the detection of a pathogenetic heterozygous variant in *NF1* [1]. The cited typical NF1 features tend to appear in an age dependent manner making clinical diagnosis uncertain in those young cases who lack pathognomonic features such as tumors or LNs. In youngers, CALMs and freckling can be the solely manifestation of the NF1 yet they, alone, are not anymore specific for NF1 [1]. In pediatric age, differential diagnosis between NF1 and LGSS becomes fundamental considering that, among RASopaties, LGSS is the one that mostly overlaps to NF1. It is due to heterozygous pathogenetic variants in *SPRED1*, and lead to a very mild phenotype with pigmentary features of NF1 [1]. In terms of prevalence LGSS occurs in about 1/46,000–1/75,000 live births, accounting for about 4% of patients followed at a neurofibromatosis clinic, whereas NF1 occurs in about 1/2000–1/3000. LGSS typically lack all tumoral NF1 features, and LNs [1]. In the least decades, only few genotype–phenotype correlations in NF1 have been reported [1]. Among these, aminoacidic substitutions of Arg1809 are related to a milder NF1 phenotype [2–4]. In 2015, Rojnueangnit et al. described the phenotypical features of the largest reported cohort of patients carrying Arg1809 substitutions [4]. The authors highlighted how fewer patients had LNs compared to other NF1 patients (6/51 in individuals ≥9 years and 10/91 in all ages) [4]. Merging data from their population with those from already published an even lower proportion of patients with LNs came out 10/119 (8.4%). Indeed, none of the previous authors did find LNs in their Arg1809 patients. One of the patients presenting LNs and reported by Rojnueangnit et al. was a 22-year-old woman with a segmental form of NF1, in whom two different somatic variants [c.5425C>T (p.Arg1809Cys) and an in-frame deletion of 228 residues c.(63_205)_(888_958)del (p.Arg69_Lys296del)] were detected in melanocytes cultured [4]. Rojnueangnit et al. did not detect any clear-cut cutaneous, dermal and/nor subdermal neurofibromas in their personal series in line with previous reports. Noonan syndrome features, including short stature, macrocephaly, and thoracic anomalies are frequently present [4]. Thus, patients with Arg1809 substitutions typically present CALMs and freckling, tend not to show typical NF1-related tumors (e.g., optic gliomas, plexiform NF) while congenital heart defects are not typically observed [4]. In the studied cohort of 7000 NF1 patients, the Arg1809 substitution occurred with a frequency of 1.23% [4].

LNs occur in more than 95% of NF1 adults and in fewer than half of those younger than 5 years of age [5]. In patients with typical CALMs, without NF1-related tumors, and lacking iris LNs, Arg1809 substitution in *NF1* or mutations in *SPRED1* should be suspected.

Besides LNs, CAs appearing as bright patchy regions detected by near-infrared (NIR) imaging have been recently identified as an additional ophthalmologic criterion for the diagnosis of NF1 [6]. The presence of two or more CAs seems highly specific to NF1 [1] and tends to be ranging from 69 to 77% of NF1 patients [6, 7]. These bright hyperreflective areas called nodules for their homology with LNs and can be revealed by optical coherence tomography (OCT) or NIR imaging [8].

The accuracy of the detection of choroidal nodules seems also higher than the presence of LNs, as reported by Viola et al. in 2012 and Vagge et al. in 2015 and [6, 7]. The authors revealed a higher frequency of choroidal nodules in the pediatric NF1 group compared to the detection rate of LNs in the same group. CAs thus tend to appear earlier than LNs and in this sense can help for the diagnosis in the first years of life.

On this background, we investigated the presence of CAs in patients with Arg1809 NF1.

We selected seven patients (5 males, median age 26.84 years, range 11–56 years) with a molecular diagnosis of NF1 and

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Arg1809 substitution, belonging to four unrelated pedigrees already reported from us [2]. All patients and their guardians gave their consent for publication. These patients underwent a complete ophthalmologic examination, including best-correct visual acuity evaluation, LNs detection by means of slit lamp examination, fundus examination, retinography, OCT, NIR, and autofluorescence imaging. None of the subjects investigated presented CAs on either OCT or NIR.

Cassiman et al. suggested that CAs are more frequently found in people with NF1 due to truncating mutations (about 71%) than those with a with a non-truncating mutation (50%) [9]. The authors also reported that never more than one nodule/eye was detected in LGSS [9].

The complete absence of the choroidal lesions in our patients confirms the genotype–phenotype association already hypothesized and suggests that the combined absence, or very low frequency, of either LNs or CAs can be characteristic of NF1 patients with aminoacidic changes affecting Arg1809. It will be fundamental in future to devote an ophthalmological study to these patients, and it might be worth to make it only on adults to ensure the fully penetrance of the trait.

As already proposed by Legius et al. [1], it is emerging that a more appropriate nomenclature is needed to rename NF1 and LGSS. Among NF1 mutated patients, even if small, a cohort exists of patients with Arg1809 substitutions, who never will experience typical NF1 tumors and typical eye findings. These patients maybe deserve a specific nomenclature of their disorder given the dramatic change in terms of phenotypical manifestations, quality of life, and life expectancy. Similar assessment should be performed among patients having the c.2970_2972del p.(Met992del) mutation. The latter mutation is known to lead to a benign NF1 phenotype under the oncological aspect, in fact patients typically lack cutaneous, sub-cutaneous, plexiform, and spinal neurofibromas and other malignant complications of NF1 while learning difficulties appear to be relatively frequent.

CAs appear earlier than Lisch nodules. Our results strongly suggest that molecular testing for Arg1809 substitution or *SPRED1* mutations should be first considered in a child around 7 years of age with just CALMs and/or freckling and without other cardinal diagnostic criteria such as OPG, neurofibromas, and bone dysplasia, as well as without either LNs or CAs. This kind of approach could make genetic test cheaper and faster respect of the classical triad NGS/MLPA/RT-PCR analysis, which ensure a higher mutation detection rate [10].

The reason why substitutions of Arginine in position 1809 lead to absence of NF1 ophthalmological typical findings is still unknown and further studies with larger cohorts of patients are needed.

DATA AVAILABILITY

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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CS conceived, wrote and revised the manuscript; GP and SP enrolled patients, wrote and revised the manuscript; FS, CI and RB conceived the work and performed ophthalmological examination, wrote and revised the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

Ethical approval was not required, because ophthalmological assessment is clinically routinely performed in patient s with NF1. Written informed consent for publication was obtained by all patients and/or their parents.

ADDITIONAL INFORMATION

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