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LETTER

Arg^{1809} substitution in neurofibromin: further evidence of a genotype–phenotype correlation in neurofibromatosis type 1

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Pinna *et al* recently proposed a novel genotype–phenotype correlation in neurofibromatosis type 1 (NF1; MIM 162200).[1] They reported six unrelated patients with the heterozygous c.5425C>T p.(Arg1809Cys) missense variant that causes a mild form of NF1, characterized by café-au-lait spots (CALS) and skinfold freckling (SF) without other typical signs of the condition such as Lisch nodules (LNs) and neurofibromas (NFs). Short stature, macrocephaly, thoracic anomalies, and other Noonan syndrome (NS) features were also present. Conversely, typical NF1-related tumors (eg, optic gliomas, plexiform NF) and congenital heart defects were not observed.[1]

After the cloning of *NF1*,[2] several studies explored the NF1 phenotype to define any genotype–phenotype correlation. In the past three decades, only two scenarios have been described: the c.2970_2972delAAT p.(Met992del) in-frame deletion and the *NF1* microdeletion, related to a benign and a very severe NF1 phenotype,[3–5] respectively. No other noteworthy correlations have been reported in NF1, although significant intrafamilial correlations for number of CALS, cutaneous NFs, and head circumference were previously reported in three large cohorts.[6,7]

We investigated the genotype–phenotype association suggested by Pinna *et al* in our cohort of 219 genetically confirmed NF1 patients monitored at our Neurofibromatosis Referral Centre. We identified the c.5425C>T substitution in three unrelated probands, who were subsequently clinically re-examined together with their affected relatives. Our results fully replicated data by Pinna *et al,* and clinical features of affected individuals are summarized in Supplementary Table 1 (Family 1–3). Informed consent was obtained from all the subjects investigated. Mutation screening was carried out at the RNA level and completed by MLPA analysis.

We also speculated about the phenotypical impact of different changes in Arg^{1809}. We therefore looked for other missense variants affecting this position. Our cohort of genetically confirmed NF1 patients also included three related patients (Family 4) and one sporadic case (Family 5) carrying the already reported c.5426G>T p.(Arg1809Leu) substitution[8] (Supplementary Table 1). In addition, a novel c.5426G>C p.(Arg1809Pro) substitution was present in a further sporadic case (Supplementary Table 1; Family 6). For sporadic patients, the *de novo* occurrence of NF1 was demonstrated by the exclusion of the mutation in children's parents. As with subjects carrying the p.(Arg1809Cys) substitution, those with a different amino acid change at position 1809 also presented a mild NF1 phenotype with some NS features. These two variants were not annotated in the Exome Variant Server or in the ExAC Browser, which altogether collect data from about 68 000 human exomes. In addition, both amino acid changes were predicted to be deleterious by common *in silico* prediction programs (SIFT, Polyphen-2 and Mutation Taster), supporting their pathogenic effect.

In the patients presented here, and those described by Pinna *et al*, substitution of the charged and basic Arg^{1809} with the hydrophilic Cys or the hydrophobic Leu and Pro amino acids seems to be always associated with a mild NF1 phenotype with only cutaneous pigmentary manifestations, without NFs and LNs, and with some NS features (Supplementary Table 1). Three-dimensional homology modeling,[9] based on the available Sec/PH-like bipartite model (RCS-PDB: 2Q4D),[10] confirmed that all the identified amino acid changes at position 1809 have the unique effect of removing the hydrogen bond between the side chain of Arg^{1809} and the backbone of Ser^{1738} (Supplementary Figure S1), as compared with the wild type (RefSeq: NP_000258.1; residues 1560–1816).

Our observations support the hypothesis that a rearrangement of the secondary structure of PH-like domain might modify the lipid-binding properties of the adjacent Sec14-like domain.[1,10] However, it remains to be clarified why this change is not sufficient to cause typical NF1 signs (eg, LNs) and why it is associated with a reduced incidence of learning difficulties, whereas it is known that close to half of the children with NF1 experience variable cognitive vulnerability.[11,12]

Five out of seven adults reported here showed at least one lipoma, suggesting that this mild NF1 phenotype might cause lipomas rather than NFs. Lipomas have been rarely reported in association with NF1[13,14] and are frequent in the general population. Thus, their occurrence could be coincidental in these patients.

The lack of a clear genotype–phenotype correlation in NF1 is still considered to be a dogma by all clinicians involved in NF1 patient care. Our observations, however, together with those published by Pinna *et al*, further support a novel association between the Arg^{1809} substitution and a mild NF1 phenotype, with a consequent impact on the prognosis, timing of follow-up, and genetic counseling.

For patients presenting with only multiple CALS, with or without SF, it may be worth prioritizing p.Met992del mutation and Arg^{1809} substitution, when a *NF1* mutation screening is carried out. *NF1* testing should obviously be combined with *SPRED1* analysis.[15]

For such observational studies, sample sizes need to be scaled up owing to the relatively low frequency of the reported variants, likely underestimated as people with mild phenotypes might never require any medical assessment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Claudia Santoro[1], Anna Maietta[1], Teresa Giugliano[2], Daniela Melis[3], Silverio Perrotta[1], Vincenzo Nigro[2,4] and Giulio Piluso*[,2]

[1]*Dipartimento della Donna, del Bambino e di Chirurgia Generale e Specialistica, Seconda Università degli Studi di Napoli, Napoli, Italy;*

[2]*Dipartimento di Biochimica Biofisica e Patologia Generale, Seconda Università degli Studi di Napoli, Napoli, Italy;*
[3]*Dipartimento di Pediatria, Università degli Studi di Napoli "Federico II", Napoli, Italy;*
[4]*Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli (NA), Italy*
E-mail: giulio.piluso@unina2.it

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