Germline mutation in *BRAF* codon 600 is compatible with human development: *de novo* V600G mutation identified in a patient with cardio-facio-cutaneous syndrome

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B-Raf, the protein product of *BRAF*, is a serine/threonine protein kinase and one of the direct downstream effectors of Ras. Somatic mutations in *BRAF* occur in numerous human cancers, while germline *BRAF* mutations cause cardio-facio-cutaneous (CFC) syndrome, a developmental disorder characterized by craniofacial dysmorphism, cardiac defects, ectodermal anomalies and developmental delay. One recurrent somatic mutation, V600E, is frequently found in several tumor types, including melanomas, papillary thyroid carcinomas, colon cancers, and ovarian cancers. Both somatic and germline *BRAF* mutations are predominantly gain-of-function mutations, leading to increased signaling through the Ras/mitogen-activated protein kinase (MAPK) cascade.

The distribution of germline CFC mutations partially overlaps those identified in human cancers but is largely unique. Somatic *Braf* mutations are generally restricted to the glycine-rich P loop or the activation segment of the B-Raf kinase domain, with the highly activating V600E mutation accounting for over 90% of these mutations. Missense mutations identified in individuals with CFC syndrome are more widely distributed, with the most prevalent mutation, Q257R, occurring within the cysteine-rich domain. Mutations affecting codon 600 have never been described in individuals with CFC syndrome, presumably because alterations of this crucial codon are not tolerated in development, which has been demonstrated in mouse models.

Here we present a patient who has features consistent with CFC syndrome and a *de novo* germline mutation involving codon 600 of B-Raf, thus providing the first evidence that a pathogenic germline mutation involving this critical codon is not only compatible with development but can also cause the CFC phenotype. *In vitro* functional analysis demonstrates that this mutation, which replaces a valine with a glycine at codon 600 (p.V600G), leads to increased ERK phosphorylation compared to wild-type B-Raf but is less strongly activating than the cancer-associated V600E mutation. The discovery of this mutation may, therefore, help to further delineate the functional difference between cancer-associated and CFC-associated *BRAF* mutations.