

Acknowledgments

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Supplementary Data

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Paternal gonadal mosaicism as cause of a puzzling inheritance pattern of activated PI3-kinase delta syndrome



Birth defects caused by de novo chromosomal abnormalities resulting in single-gene defects occur in approximately 5% of all live births.¹ Affected offspring have a broad range of illnesses, including primary immunodeficiency diseases, a genetically heterogeneous group of disorders presenting with a clinical spectrum of mild to severe infections, often complicated by autoimmunity and malignancies.² Most primary immunodeficiency diseases are monogenic diseases with a Mendelian inheritance pattern.²

Recently, heterozygous gain-of-function mutations in *PIK3CD*, encoding the catalytic p110 δ subunit of phosphatidylinositol 3-kinase (PI3K), were identified in patients presenting with recurrent respiratory tract infections, hepatosplenomegaly, polyadenopathy, CD4 lymphopenia, and decreased numbers of class-switched memory B cells, a phenotype referred to as activated PI3K delta syndrome type 1 (APDS1).^{3,4} Patients with APDS1 exhibit increased basal and stimulation-dependent activation of the AKT-mTOR pathway. Similar clinical findings and PI3K signaling activation have been observed in patients with heterozygous mutations in the *PIK3R1* gene, encoding the regulatory subunit of PI3K, referred to as APDS2.⁵

We identified a kinship that included 3 half-siblings with symptoms typical for APDS1. The index case (II.4 in Fig 1A) had 10 episodes of pneumonia, 2 episodes of sepsis, several upper respiratory infections, and oral moniliasis within the first year of life. He subsequently developed hepatosplenomegaly, lymphadenopathy,

and an axillary abscess owing to *Candida albicans*. At 3 years of age, laboratory investigation showed increased immunoglobulin (Ig) M (368 mg/dL) and IgG (1,450 mg/dL) levels, normal IgA level (107 mg/dL), low CD4 (330/mm³) and increased CD8 (1,229/mm³) counts, and low CD19 B cells (17/mm³). IgG subclasses showed normal absolute levels of IgG1 (1,020 mg/dL), IgG2 (79.0 mg/dL), IgG3 (78.3 mg/dL), and IgG4 (28.1 mg/dL); however, their ratio showed a proportional decrease of IgG2.

Based on the clinical symptoms, APDS1 was suspected and a heterozygous *PIK3CD* hotspot mutation (c.3061G→A, p.E1021K) was identified by Sanger sequencing. He was treated with intravenous immunoglobulin, cotrimoxazole prophylaxis, and rapamycin, resulting in marked decrease of hepatosplenomegaly and alleviation of his other symptoms.

The patient's father (I.4), a truck driver, reported that in addition to the index case, he had 4 additional children with 3 other women living in different Brazilian cities along his truck route and that 2 of these children (II.4 and II.5) had symptoms similar to the index case (Fig 1A). Of his 5 children, 1 had died at 3 years of age (II.1) with clinical symptoms similar to the index case, including hepatosplenomegaly, fever, and recurrent infections; immunologic studies were not performed. The other symptomatic half-brother (II.5) was evaluated at 5 years of age with a history of 5 episodes of pneumonia, recurrent oral candidiasis, several upper respiratory infections, and hepatosplenomegaly. He had increased IgM (219 mg/dL) and IgG (1,550 mg/dL) levels, normal IgA levels (206 mg/dL), low CD4 (460/mm³) and CD8 (247/mm³) counts, and normal CD19 counts (300/mm³). Sanger sequencing identified the same heterozygous *PIK3CD* hotspot mutation (p.E1021K) found in his half-

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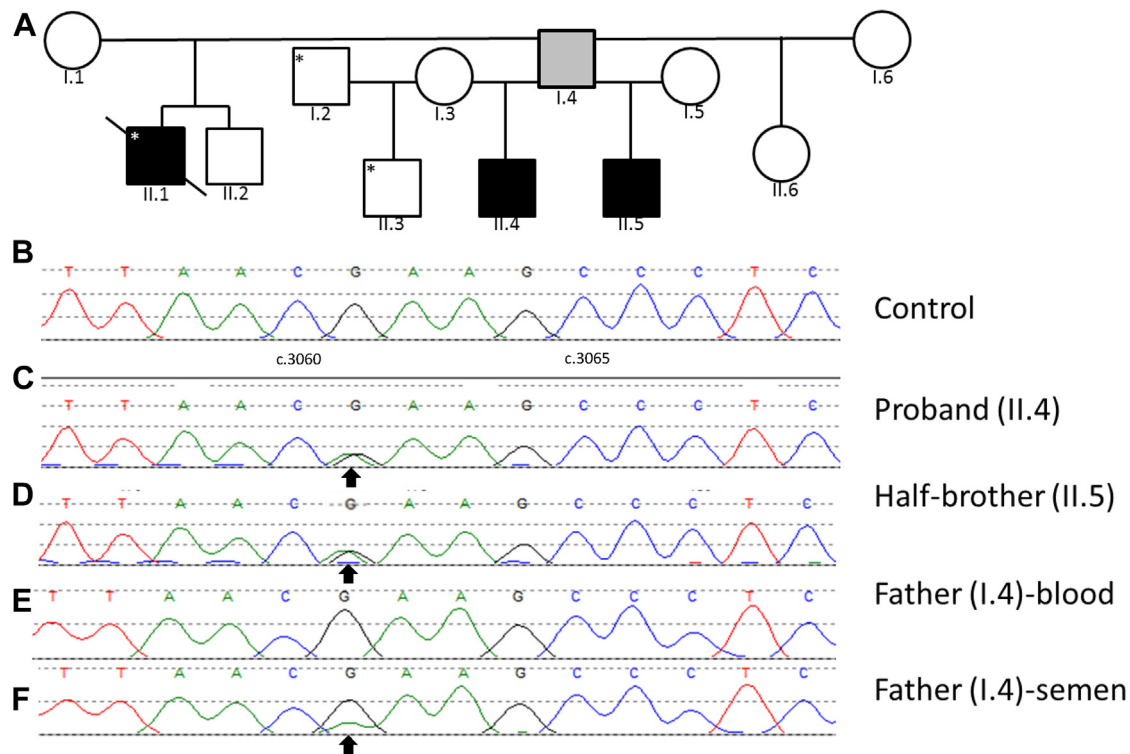


Figure 1. (A) Family pedigree. (B–F) Molecular sequence of the catalytic p110 δ subunit of phosphatidylinositol 3-kinase showing the heterozygous mutation G $\rightarrow$ A at position c.3061 (black arrows) in the proband and his half-brother and in genomic DNA from their father's semen but not in his blood. I.4, non-affected father; I.1, I.3, I.5, I.6, non-affected mothers; II.1, II.4, II.5, affected half-brothers; II.2, II.3, II.6, non-affected half-brothers. *Subjects not tested for p.E1021K mutation.

brother, the index case (Fig 1C, D). He was treated successfully with intravenous immunoglobulin and cotrimoxazole prophylaxis.

The pedigree suggested that the father (I.4) carried the same autosomal-dominant *PIK3CD* mutation that affected 3 sons born to different mothers. However, neither he nor the mothers of the affected boys had any symptoms suggestive of APDS. Sanger sequencing demonstrated that neither the father nor the mothers of the affected boys carried the identified *PIK3CD* mutation in blood. This raised the possibility of germline or gonadal mosaicism in the father. To test this hypothesis, genomic DNA was extracted from his semen. As illustrated in Figure 1F, semen-derived DNA carried the heterozygous p.E1021K mutation identified in the affected sons. Based on relative peak heights, we estimated that 20% to 25% of the semen carried the mutation.

Germline or gonadal mosaicism, in which gametes harbor genetic variants that are not present in other tissues, results from somatic mutations that arise at some point during the development of the reproductive organs.¹ The point at which the mutation occurs dictates how many of the resulting gametes will ultimately harbor the variant and thus the resulting risk for having affected offspring. Gonadal mosaicism of the mother and of the maternal grandfather has been inferred based on pedigree studies in various X-linked recessive immunodeficiency disorders, including X-linked severe combined immune deficiency, Wiskott-Aldrich syndrome, X-linked ectodermal dysplasia, anhidrosis, and immune deficiency.^{6–9} To our knowledge, this is the first report of experimentally proved paternal gonadal mosaicism in an inherited immunodeficiency disorder, although paternal gonadal mosaicism has been reported in other genetic disorders. It is assumed that this variant arose de novo during the development of the father's gonadal tissue because the mutation was not observed in DNA derived from peripheral blood. De novo germline mutations in *PIK3CD* as a cause of APDS1 have been observed in approximately 30% of affected families.⁴ The Sanger sequencing method might not be sensitive enough to detect

mosaicism in fewer than 5% to 10%, so we cannot rule out the possibility that the father might have had low-level mosaicism in peripheral blood that was below the limit of detection. Incomplete allele penetrance has been described in patients with APDS, but we attributed the absence of symptoms in the father to the absence of immune cell mutation. Because the p.E1021K variant is considered a gain-of-function mutation in a key signaling molecule critical for lymphocyte growth and development, we hypothesize that even if the father had a low level of somatic mosaicism, these cells should have a selective growth advantage and would likely have expanded to occupy an ever growing proportion of the father's peripheral blood leukocytes, causing him to exhibit symptoms of APDS and allowing the mutation to be detected by sequence analysis, which it was not.

Interestingly, although the mutant allele appeared to be present in only 20% to 25% of sperm, 3 of the father's 5 children were affected by APDS1. Advanced paternal age has been associated with an increased risk of autosomal dominant disorders in offspring resulting from de novo mutations arising in spermatogonia.^{1,10} It has been hypothesized that some of these mutations, such as activating mutations in the Ras–mitogen-activated protein kinase pathway in Noonan syndrome, can lead to a selective survival or functional advantage of the mutated sperm, a process termed “selfish spermatogonial selection.”¹⁰ Because the evaluation of reproductive capacity has largely been ignored in the setting of germline signaling defects observed in patients with immunodeficiency, we suggest a similar approach to investigate the possibility of gonadal mosaicism in clinically normal fathers of offspring with de novo gain-of-function mutations.

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Bee moth (*Galleria mellonella*) allergy New insight from a case of monosensitization



We present a case of bee moth (*Galleria mellonella*) allergy with new clinical and immunologic features. While taking part in trout fishing games during the past year, a 34-year-old amateur fisherman with no personal history of allergic diseases experienced several attacks of rhinoconjunctivitis and late asthmatic reactions. Symptoms of rhinoconjunctivitis began when starting to fish and persisted during the day. This was followed by mild wheezing and dyspnea during the following night. Symptoms worsened during the most recent fishing excursion, and mild breathing difficulty tends to last until the night of the next day. In the latest episode, he had a strong asthma attack during the night, which required treatment in the emergency department with intravenous steroids and salbutamol spray. The results of skin prick tests (SPTs) and IgE dosing for a large panel of airborne allergens were negative.

We later performed a prick-by-prick test with living bee moth larvae, the bait the patient used for trout fishing. The test produced a strong positive reaction (irregularly outlined wheal area, 34 × 9 mm). Although the histamine skin reaction faded gradually, the *G mellonella* site had a progressive increase of edema and erythema, which peaked at 6 to 8 hours, when it broadened to approximately half of the forearm, lasting with no significant changes up to 12 hours. Eighteen hours after the test, the skin reaction had gradually decreased, but at 24 hours a red papule surrounded by a relatively small area of pale erythema and edema was clearly evident. After 2½ days, a small papule on a little palpable area of indurate skin was still visible.

Because a previous study found no reactivity to heat-treated *G mellonella* larvae (mummies),¹ we performed a prick-by-prick test with a mummified, uncolored larva. Surprisingly, the test elicited a strong immediate response (wheal area, 13 × 10 mm) followed by a late-phase reaction similar to that seen with the living larva, which disappeared after approximately 30 hours. Prick-by-prick tests performed with both live and mummified larvae in 2 non-allergic controls had negative results.

A challenge test was performed simply by opening a commercial package that contained 35 live baits in the presence of the patient. A honey-like smell spread out in the room, causing immediate symptoms of rhinoconjunctivitis in the patient. No symptoms were triggered with a box of mummified larvae, and fishing with mummies did not elicit any symptoms.

To study the allergens responsible for the reaction, proteins were extracted from both raw and heat-treated larvae with phosphate-buffered saline (soluble proteins) and with a denaturing buffer (total proteins). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis in reducing conditions and immunoblotting analyses were performed as previously described.² IgE and IgG (subclasses 1, 3, and 4) binding was revealed by specific secondary antibodies. The serum of a healthy individual was used as the control (not shown). Immunoblotting analysis of raw *G mellonella* revealed a strong IgE-binding band with a mobility of approximately 27 kDa in both the soluble and total protein extracts, which could correspond to the hemolymph glycoprotein, previously identified as a bee moth allergen.³ Another band was detected in both the soluble and total protein extracts, where found mobilities of 45 and 40 kDa, respectively. In contrast, the analysis of heat-treated *G mellonella* revealed a single IgE-binding band of approximately 20 kDa, suggesting that cooking the larvae changes protein extractability and pattern (Fig 1). This latter band was found in the total protein extract but not in that obtained with phosphate-buffered saline (soluble protein). This could be why no thermostable allergens were found in previous study.¹ Despite the presence of IgE, it is not surprising that handling and fishing with bee moth mummies did not challenge respiratory symptoms because they seem to be caused by inhalation of living insect emanations.

Skin late-phase reaction (LPR) to bee moth and others insects used as fish bait has never previously been reported. In our case, the development of the skin LPR was continuous with the immediate reaction (not biphasic) and elicited by prick tests with both alive and heat-treated larvae. Skin LPRs are usually induced by intra-dermal injection (not by SPT) of sufficiently high concentrations of allergen. The inflammatory process demonstrates itself to be