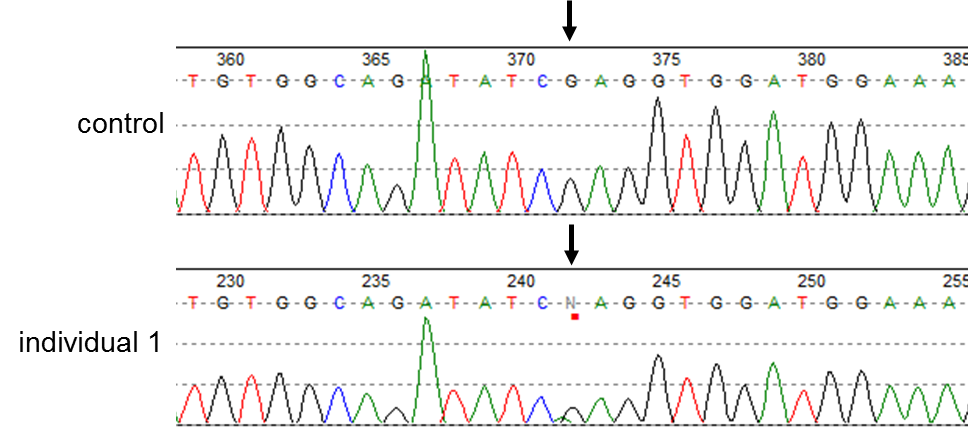
**Supplemental Data**

**Supp. Figure S1: Electropherograms of the identified *RHOA* c.139G>A variant**.

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**Legend:** PCR and subsequent Sanger sequencing confirmed the *RHOA* c.139G>A (RefSeq NM\_001664.4) variant in DNA extracted from skin biopsy of individual 1 (lower panel) and its absence in control DNA (upper panel). c.139 position in *RHOA* is indicated by black arrow.

**Supp. Table S1: Deep-Sequencing results for the *RHOA* c.139G>A on DNA isolated from blood, skin biopsy and buccal swab of individual 1, and control DNA derived from blood.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** |  | **Total number of reads** | **T** | **G** | **C** | **A** | **del** |
| Individual 1 (blood) | read count | 1,2 10\*6 |  |  |  |  |  |
|  | % |  | 0.05 | 99.86 | 0.05 | 0.05 | 0.00 |
| Individual 1 (skin biopsy) | read count | 1,2 10\*6 |  |  |  |  |  |
|  | % |  | 0.02 | 74.32 | 0.05 | 25.62 | 0.00 |
| Individual 1 (buccal swab) | read count | 1,1 10\*6 |  |  |  |  |  |
|  | % |  | 0.03 | 99.94 | 0.00 | 0.03 | 0.00 |
| control DNA (blood) | read count | 1,2 10\*6 |  |  |  |  |  |
|  | % |  | 0.01 | 99.99 | 0.00 | 0.00 | 0.00 |

**Supp. Table S2: Deep-Sequencing results for the *RHOA* c.139G>A on DNA isolated from skin biopsy of individual 2, and parental saliva samples.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** |  | **Total number of reads** | **T** | **G** | **C** | **A** | **del** |
| Individual 2  (skin biopsy) | read count | 110736 | 26 | 92291 | 9 | 9205 | 0 |
|  | % |  | 0.0 | 83.3 | 0.0 | 8.3 | 0.0 |
| Father  (saliva) | read count | 122634 | 19 | 122568 | 8 | 39 | 0 |
|  | % |  | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 |
| Mother  (saliva) | read count | 89680 | 8 | 89612 | 10 | 50 | 0 |
|  | % |  | 0.0 | 99.9 | 0.0 | 0.1 | 0.0 |

**Supp. Table S3: Deep-Sequencing results for the *RHOA* c.139G>A on DNA isolated from blood and skin biopsy of individual 3, and parental blood samples.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** |  | **Total number of reads** | **T** | **G** | **C** | **A** | **del** |
| Individual 3 (blood) | read count | 8382 | 4 | 8370 | 4 | 4 | 0 |
|  | % |  | 0.05 | 99.86 | 0.05 | 0.05 | 0.00 |
| Individual 3 (skin biopsy) | read count | 11056 | 2 | 8217 | 5 | 2832 | 0 |
|  | % |  | 0.02 | 74.32 | 0.05 | 25.62 | 0.00 |
| Father  (blood) | read count | 18008 | 5 | 17998 | 0 | 5 | 0 |
|  | % |  | 0.03 | 99.94 | 0.00 | 0.03 | 0.00 |
| Mother (blood) | read count | 9109 | 1 | 9108 | 0 | 0 | 0 |
|  | % |  | 0.01 | 99.99 | 0.00 | 0.00 | 0.00 |

**Supplemental Material and Methods**

**RHOA expression construct**

Generation of expression wild-type RHOA (RefSeq NM\_001664.4; NP\_001655.1) expression plasmid containing coding sequences of human *RHOA* with an additional, N-terminal FLAG tag was amplified by RT-PCR from isolated HEK293T RNA and cloned into the pcDNA3 expression vector (Life Technologies, Germany). c.139G>A variant was introduced by site directed mutagenesis and all cDNA sequences were confirmed by Sanger sequencing.

**Cell culture and immunoblotting**

HEK293T cells were cultured in Dulbecco’s modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal calf serum (FCS, Gibco), and antibiotics. Transfection was carried out with 2 µg of each plasmid by using Fugene® HD Transfection reagent (Promega, Germany) following manufacturer’s instructions, and cells were solubilized 24 hours post transfection.

**Protein isolation and Western blot analysis**

HEK293T cells were solubilized by using ice-cold RIPA buffer (10 mM Tris, pH: 8.0; 150 mM NaCl; 1 mM EDTA; 10 mM NaF; 1 mM Na3VO4; 10 µM Na2MoO4; 1% NP-40; protease inhibitors P 2714 [Sigma-Aldrich, USA]). The total protein concentration of extracts was determined using the BCA Protein Assay Kit (Thermo Fisher Scientific, USA), and 20 µg of total cell lysates were separated by SDS-Polyacrylamide gel electrophoresis. Transfer and immunoblotting were performed according to standard protocols. Anti-β-Actin and anti-FLAG antibodies were purchased from Sigma-Aldrich. Secondary antibodies conjugated to peroxidase (Santa Cruz Biotechnology Inc., USA) were used and blots were developed using an enhanced chemiluminescence system (Bio-Rad, USA), followed by detection using the ChemiDocTM Touch Imaging System (Bio-Rad, USA).

**Amplicon-based deep sequencing**

PCR-amplification on the targeted DNA sequences was performed using custom designed primers. PCR products were then used for Illumina TruSeq DNA library preparation without introducing further amplification steps and subsequently sequenced on a Illumina HiSeq4000 sequencer with a PE75 protocol.

**Structural analysis and *in silico* prediction**

Structure of the GTP-bound form of RHOA in complex with the effector domain of PKN1 were obtained from the Protein Data Bank (www.wwpdb.org; PDB 1CXZ). The RHOA-PKN1 complex comprises of RHOA (residues 1–181) and PKN1 (residue 13–98), and structural analysis and figure preparation were carried out with the program PyMOL 2.3 (www.pymol.org; Schrödinger, LLC). *In silico* prediction of the potential effect of the identified variant c.139G>A in *RHOA* was performed using SIFT (http://sift.bii.a‐star.edu.sg/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), CADD (https://cadd.gs.washington.edu), and MutationTaster (http://www.mutationtaster.org). Protein alignment was performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo).