Functional tests to guide management in an adult with loss of function of type-1 angiotensin II receptor

Supplementary material

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Supplementary methods

**DNA preparation and next-generation sequencing**

The original samples were submitted to Otogenetics Corporation (Atlanta, GA USA) for human exome capture and sequencing. Genomic DNA (gDNA) was isolated from whole blood using the FlexiGene DNA Kit (cat. 51204, Qiagen). Briefly, gDNA was subjected to agarose gel and OD ratio tests via Nanodrop to confirm the purity and concentration prior to Bioruptor (Diagenode, Inc., Denville, NJ USA) fragmentation. Fragmented gDNAs were tested for size distribution and concentration using an Agilent Tapestation 2200. Illumina libraries were made from qualified fragmented gDNA using New England Biolabs DNA Library Prep Master Mix Set for Illumina (New England Biolabs. Ipswich, MA USA, catalog# 6040L) and the resulting libraries were subjected to exome enrichment using SureSelectXT human All Exon V4 (Agilent Technologies, Wilmington, DE USA, catalog# 5190-4641) following manufacturer’s instructions. Enriched libraries were tested for enrichment by qPCR and for size distribution and concentration by an Agilent Bioanalyzer 2100. The samples were then sequenced on an Illumina HiSeq2000 which generated paired-end reads of 100 nucleotides (nt). Data was analyzed for data quality using FASTQC (Babraham Institute, Cambridge, UK).

**Bioinformatics Analysis and Pipeline**

Sequence reads were aligned to the Human Genome Reference Assembly GCRh37/hg19 using Burrows-Wheeler Alignment (BWA) version 0.7.12, and indexed using SAMtools version 1.6. SNVs and indels were subsequently called by the Genome Analysis Toolkit (GATK) HaplotypeCaller version 3.4-46. Variant annotation and de novo analysis were performed using a custom-designed in-house analysis pipeline.

Genotypes were compared to population frequencies as reported by gnomAD v2.1.1, the EVS data release ESP6500SI-V2 (NHLBI GO ESP Exome Variant Server) and an in-house database. Filtering was done with the filtering steps described in Supplementary table 1. Pathogenicity of the variant was assessed using the Association for Clinical Genomic Science (ACGS) Best Practice Guidelines for Variant Classification 2019 (https://www.acgs.uk.com/quality/best-practice-guidelines/, ratified 06 May 2019).

To confirm or exclude a genetic defect in the renin-angiotensin-aldosterone system, we also looked specifically for any rare variants (i.e. < 0.005 minor allele frequency) in CYP11B2, REN, ACE, AGT and AGTR1 in the unfiltered data.

**References**

1. Li, H. & Durbin, R. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* **26**, 589-595, doi:10.1093/bioinformatics/btp698 (2010).
2. Li, H. *et al*. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078-2079, doi:10.1093/bioinformatics/btp352 (2009).
**Supplementary Table 1. Filtering strategy**

This table describes the filtering steps taken after obtaining the exome sequencing data, as well as the number of variants left at each step. After filtering steps 1-3, we took two different approaches to look for causative variants, resulting in Supplementary Tables 2 and 3. We also looked for homozygous and compound heterozygous variants, resulting in the variants listed in Supplementary 3.

| Step | Filter                                           | # of variants in index | Notes                                           |
|------|--------------------------------------------------|------------------------|-------------------------------------------------|
| 0    | Before filtering                                 | 94282                  |                                                 |
| 1    | SNP frequency of in-house databases <0.5%        | 2077                   |                                                 |
| 2    | Keeping only variants in coding regions and splice sites | 625                   |                                                 |
| 3    | Excluding synonymous variants                    | 408                    | Steps 4.1 and 4.2 have been performed separately |
| 4.1  | Possible de novo variants                        | 8                      | Supplementary table 2                           |
| 4.2  | Keep only homozygous, hemizygous and compound heterozygous variants | 28                    | Supplementary table 3                           |

**Supplementary Table 2. Candidate variants, de novo**

After following the filtering steps described in Supplementary Table 1, we looked for possibly *de novo* pathogenic variants.

**Supplementary Table 3. Candidate variants, homozygous, hemizygous and compound heterozygous**

After following the filtering steps described in Supplementary Table 1, we looked for homozygous, hemizygous and compound heterozygous variants.