Clinically relevant results within the research project "Genetics of Neurodevelopmental Disorders"

Trio-exome-sequencing of

Index Mustermann, Max, 01.02.2013 ♂

Mutter Mustermann, Marie, 02.03.1988 ♀

Vater Mustermann, Mark, 03.04.1984 ♂

Diagnostik aus EDTA-Blut vom 20.02.2018 Laboreingang am 20.05.2018

Indication: neurodevelopmental disorder

Symptoms: development delay, intellectual disability, epileptic encephalopathy, seizures, autistic features, microcephaly, optic atrophy, atrophy of the corpus callosum, EEG abnormalities

Family history: parents are healthy and not related

Results: Max has probably the Bosch-Boonstra-Schaaf optic atrophy syndrome (#615722)

Due to a likely pathogenic, de novo variant in the NR2F1 gene

Summary of identified variants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variants</th>
<th>Zygosity</th>
<th>MAF*</th>
<th>Disorder (OMIM)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR2F1</td>
<td>chr5:92921166_92921167delinsAT; NM_005654.5; c.437_438delinsAT; p.(Cys146Tyr)</td>
<td>heterozygous, de novo</td>
<td>0</td>
<td>Bosch-Boonstra-Schaaf optic atrophy syndrome (#615722)</td>
<td>likely pathogenic</td>
</tr>
</tbody>
</table>

* MAF: minor allele frequency in GnomAD database

Interpretation

In the context of our research study to identify the genetic causes of neurodevelopmental disorders, we obtained results that are probably of clinical relevance.

We identified a heterozygous, de novo, indel variant, c.437_438delinsAT: p.(Cys146Tyr) in NR2F1. This variant has not been reported in general population and also not in mutation databases. In silico programs predict a pathogenic effect of the variant. Taken together, we classify this variant as likely pathogenic based on the ACMG criteria (Richards et al., 2015, PMID 25741868).

The NR2F1 gene encodes a conserved nuclear receptor protein that regulates transcription. Pathogenic variants in this gene lead to Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS), which is an autosomal-dominant disorder characterized by optic atrophy and intellectual disability, and that is caused by loss-of-function variants in NR2F1. In addition to visual and cognitive deficits, some individuals with BBSOAS manifested muscular hypotonia, seizures, autism spectrum disorder, oromotor dysfunction, thinning of the corpus callosum, and hearing defects.

Taken together, we conclude that the variant in NR2F1 is probably the cause of the symptoms of Max.
Recommendations

We recommend performing retrospective phenotypical analyses in order to evaluate the correlation between the identified variant and the symptoms of your patient. Also, we recommend validation using Sanger sequencing. We recommend notifying us about any changes in clinical presentation or new symptoms because these may have an impact on our assessment. According to § 10 of the German Genetic Diagnostic Law (GenDG), a genetic counseling should be offered to communicate the genetic findings.

We have contacted Dr. Expert von Diesem-Feld (New York, New York, USA), who is an expert for this syndrome. We are going to contribute anonymized clinical data of Max to be added to a central database. Also, a conference of the families of children with BBSOAS is going to take place in April 2019. Possibly, the family of Max is interested to participate.

Incidental findings

We have not found any pathogenic or likely pathogenic variants in the actionable genes. Please see Point 4 in additional information at the end of the report for more details.

In case there are any further questions, please do not hesitate to contact us.

Best regards

Prof. Dr. med. J. Lemke
FA für Humangenetik
Kommissarischer Leiter

Dr. rer. nat. J. Hentschel
Wissenschaftliche Mitarbeiterin
Laborleiterin Molekulargenetik

PD Dr. med. R. Abou Jamra
FA für Humangenetik
Oberarzt

B. Büttner
cand. med.
General aspects of exome sequencing

Whole-exome sequencing (WES) is a method based on the technology of massive parallel sequencing, also called next generation sequencing (NGS). This method analyzes the majority of coding sequences, the exons. The proportions of the covered exons depend on the used kit and on the quality of the analysis.

The results can be only interpreted in the context of the patient’s medical evaluation, family history, and scientific literature. Please note that the variant’s classification may change over time, as more information becomes available.

Methods and additional information

1. Information regarding Quality
   
   Q30-values: 93.16%, more detailed information can be provided on demand.
   
   Coverage of 10x has been achieved in 98.8% (index), 98.8% (mother), and 99.0% (father) of targeted sequence

2. Applied methods
   
   a. Acting on our instructions, the exome capture was carried out with Agilent SureSelect All Human Version 6 (60Mb) and the library was then sequenced on a HighSeq sequencer (Illumina) at CeGaT’s laboratory in Tübingen.
   
   b. Analysis of the rough data was performed using the software Varfeed (Limbus, Rostock) and the variants were annotated and prioritized using the software Varvis (Limbus, Rostock). We have prioritized all potential protein-influencing variants with regard to their pathogenicity and clinical relevance according to all possible inheritance modes.
   
   c. We report only variants that are located in valid disease associated genes based in OMIM. Exceptions are variants in genes of which we have other sources of information that strongly support disease-gene association. You will not be informed about variants with no clear clinical relevance.

3. Limitations of exome-sequencing
   
   a. We cannot guarantee complete coverage of all possible variants by this method due to: a) the kit and the technique do not cover all targeted sequences, b) many variants are not present in the targeted sequences, and c) some variants, e.g. larger deletions and insertions, cannot be detected with NGS.
   
   b. We evaluate variants with a minimum coverage of 10x.
   
   c. The reported variants are NOT validated with Sanger. A Sanger validation is necessary before the results influence a clinical decision. We recommend validation of the variant and segregation analysis using Sanger sequencing. Our institute may run these analyses on clinically routine basis if this is wished by you.
   
   d. We evaluate and report the data based on the available clinical information. However, a final conclusion on the relevance of the variants can only be made by the referring physician after a detailed assessment of clinical symptoms and genetic results.
   
   e. We assessed and reported the data based on the current literature. We may carry out re-evaluations of data in the future. If we end up with a significantly deviating result, we will inform you. There is no claim to re-evaluate the data.
   
   f. As mentioned in (a), larger deletions and insertions (~10bp or longer) are currently not reliably detected by exome sequencing. In the contest of this research project it is planned to carry out such analysis. If reliable and relevant results are obtained, we would inform you.

4. Information about incidental findings
   
   a. Exome-sequencing may lead to the identification of variants of medical significance that are not associated with the individual’s indication. These findings are called incidental findings and are not the subject of the investigation.
   
   b. If desired and consented by the patient or her/his guardian, we report only pathogenic and likely pathogenic (classes 1 and 2) variants in only so-called actionable genes according to the recommendation of the ACMG. Actionable genes are those which, if mutated, could lead to treatable or preventable diseases or recommendations for specific screening programs. These are currently the following genes ACTA2, ACTC1, APC, APOB, ATP7B, BMPR1A, BRCA1, BRCA2, CACNA1S, COL3A1, DSC2, DSG2, DSP, FBN1, GLA, KCNH2, KCNQ1, LDLR, LMNA, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PCSK9, PKP2, PMS2, PKRAG2, Pten, RB1, RET, RYR1, RYR2, SCNSA, SDHA, SDHb, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFB1, TGFB2, TMEM43, TNNT2, TP53, TPM1, TSC1, TSC2, VHL, and WT1.