|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Proband 2** | | | | | |
| **Gene** | **Position** | **GnomAD freq.** | **ID** | **Effect** | **Disease / Comments** |
| **Potentially AR (homozygous or compound heterozygous variants <0.01)** | | | | | |
| *TNIK*  (MIM: 610005) | chr3:170875246-C>T | 0,000077926 | rs552084095 | NM\_001161560.1:c.1222+3G>A | No known disease association. shown to be *in cis* by a family study. Frequency too high for a dominant effect. |
| chr3:170945991-C>T | 0 | - | NM\_001161560.1:p.Gly48Asp |
| **Potentially AD (predicted loss off function, freq.=0 in all available databases)** | | | | | |
| *CSNK1E*  (MIM: 600863) | chr22:038699187-T>C | 0 | - | NM\_001289912.1:p.Gln48Arg | No known disease association/ inherited from healthy parent |
| *FASN*  (MIM: 600212) | chr17:080039187-C>T | 0 | - | NM\_004104.4:p.Ala2150Thr | No known disease association / inherited from healthy parent |
|  | | | | | |

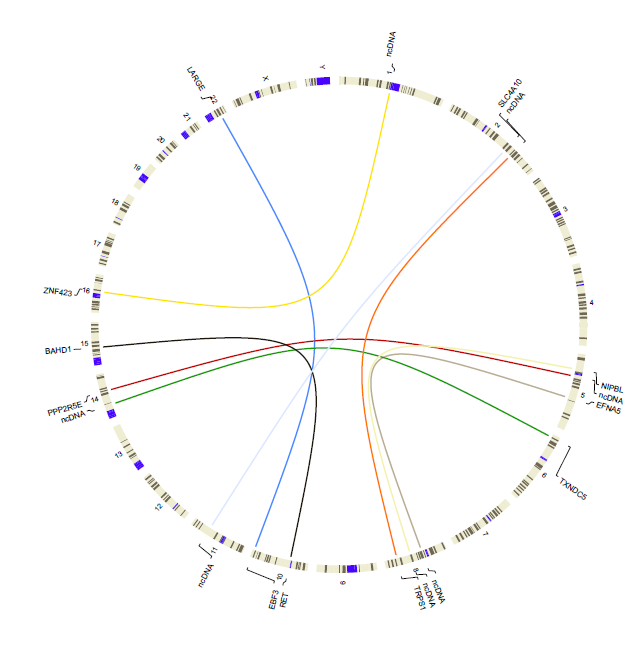
**Supplementary table 1** Potentially pathogenic variants chosen from WES results in proband 2

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Proband 5** | | | | | | | |
| **Gene** | **Position** | **GnomAD freq.** | **ID** | | **Effect** | | **Disease / Comments** |
| **HGMD hits** | | | | | | | |
| *CPA6* (MIM: 609562) | chr8:068396042-C>T | 0,0022 | | rs61738009 | | NM\_020361.4:p.Gly267Arg | Epilepsy, familial temporal lobe, 5 (AR, AD)/Febrile seizures, familial, 11 (AR), shown to be *in cis* by a family study. Frequency too high for a dominant effect. |
| chr8:068419039-G>C | 0,0015 | | rs35993949 | | NM\_020361.4:p.Gln207Glu |
| **Potentially AR (homozygous or compound heterozygous variants <0.01)** | | | | | | | |
| *MTCL1* (MIM: 615766) | chr18:008798156-C>T | 0,00014 | | rs141478791 | | NM\_015210.3:p.Ala768Val | No known disease association,  shown to be *in trans* by a family study |
| chr18:008784246-C>T | 0,000025 | | rs746035252 | | NM\_015210.3:p.Ala379Val |
| **Potentially AD (predicted loss off function, freq.=0 in all available databases)** | | | | | | | |
| *SPTBN1* (MIM: 182790) | chr2:054839299-C>G | 0 | | - | | NM\_003128.2:p.Pro101Arg | No known disease association/ inherited from healthy parent |
| *PTCH1* (MIM: 601309) | chr9:098248120-C>A | 0 | | - | | NM\_000264.3:p.Arg144Leu | Basal cell nevus syndrome/ Holoprosencephaly/ inherited from healthy parent |
| *PSPC1* (MIM: 612408) | chr13:020346550-T>C | 0 | | - | | NM\_001042414.2:p.Asn169Ser | No known disease association/ inherited from healthy parent |

**Supplementary table 2** Potentially pathogenic variants from WES results in proband 5

**Supplementary table 3** Characteristics of genes disrupted by BCT in studied probands. pLI – score from the ExAC database1 (the higher the score, the less tolerant the gene to loss-of-function mutations); DOMINO P(AD)2 – a score derived from machine learning to predict genes associated with dominant disorders, the higher the score, the higher the probability that the gene causes a disease with autosomal dominant inheritance). AD - autosomal dominant, AR - autosomal recessive.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Proband | Gene | OMIM#, Inheritance | ExAC pLI | DOMINO P(AD) |
| 1 | *EFNA5* (MIM: 601535) | unknown | 0,89 | 0,99 |
| 2 | *SLC4A10* (MIM: 605556) | unknown | 0,0 | 0,20 |
| 3 | *BAHD1* (MIM: 613880) | unknown | 0,99 | 0,63 |
| 3 | RET (MIM: 164761) | AD | 1,00 | 1 |
| 4 | *PPP2R5E* (MIM: 601647) | unknown | 1,00 | 0,86 |
| 5 | *TXNDC5* (MIM: 616412) | unknown | 0,00 | 0,14 |
| 5 | *BLOC1S5-TXNDC5* (NA) | unknown | - | 0,052 |
| 6 | *EBF3* (MIM: 607407) | AD | 1,00 | 0,99 |
| 6 | *LARGE* (MIM: 603590) | AR | 0,96 | 0,83 |
| 7 | *ZNF423* (MIM: 604557) | AR;AD | 0,99 | 0,98 |
| 8 | *NIPBL* (MIM: 608667) | AD | 1,00 | 1 |
| 9 | *TRPS1* (MIM: 604386) | AD | 0,99 | 0,97 |

**Supplementary figure 1** A circos plot containing all the translocations in all the probands. Each color represents a single patient. ncDNA - non-coding DNA.

**SUPPLEMENTARY METHODS**

**Visualization of Topologically Associating Domains (TADs) in the region of brakpoints**

TADeus web service (available on http://bioputer.mimuw.edu.pl/tadeus) was used to generate plots showing genomic region spanning ±1,5Mb from every translocation breakpoint. The chromatin interactions were visualized as a color-coded heatmap, where both axes represent loci in the order of the genome and the colour of each pixel corresponds to the number of observed interactions between two loci standardized using iterative correction and eigenvector decomposition (ICE)3. Such matrices are by definition symmetric around the diagonal, and the number of rows and columns is equal to the length of the genome divided by the bin size. The visualization used shows the upper submatrix with x- axis representing the diagonal. Moreover TADs are depicted as red triangles. Every plot includes Hii-C data from human GM12878 B-lymphoblastoid cells4. Data in .cool format were downloaded from ftp://cooler.csail.mit.edu/coolers/hg19/}. Topologically Associating Domains (TADs) were detected using Insulation Score method5. For this purpose matrix2insulation.pl Perl script from https://github.com/dekkerlab/crane-nature-2015 with parameters: is=500000 ids=200000 im=mean bmoe=3 nt=0.1 was used. Below genes located in the region are shown with transcription direction indicated by an arrow. The genes are color-coded according to their pLI score and DOMINO score.

**SUPPLEMENTARY DISCUSSION**

**Probands with translocations in genes with known function**

In Proband 6 the BCTs disrupted genes *EBF3* (MIM: 607407)and *LARGE* (MIM: 603590)*.* *EBF3* is associated with Hypotonia, ataxia, and delayed development syndrome (MIM: [617330](https://omim.org/entry/617330)) 6-10 which well correlated with the patient’s phenotype. The *EBF3* associated disease is inherited in an autosomal dominant mode which further supports the causative role of *EBF3* disruption in patient’s disease. Defects of *LARGE* cause muscular dystrophy-dystroglycanopathy (MIM: [613154](https://omim.org/entry/613154))11. Given the symptoms of this disease as well as its autosomal recessive inheritance we think that monoalleic disruption of *LARGE* had no impact on the phenotype of the patient.

In proband 7 the *ZNF423* (MIM: 604557)gene has been disrupted. The proband was a girl born at 41 weeks of the first pregnancy with an Apgar score of 10, weight 3090 g (25-50th centile), height 52 cm (90th centile), and head circumference 34 cm (50th centile). A healthy sister was subsequently born from GIIPII. In the newborn period, severe failure to thrive and distal symphalangism of the left thumb were observed. Both thumbs were hypoplastic.

She sat at 8 months, began to walk at 16 months, and delayed speech development was noted. At the age of 1 17/12 years she weighed 8.1 kg (<3rd centile) with a height of 79.6 cm (25th centile). At this age, generalized hypotonia, poor motor coordination and delayed speech (single words) were noted.

At last examination, at 3.5 years of age, her anthropometric parameters were: weight 11 kg (<3rd centile), height 94 cm (3rd centile), OFC 54 cm (75-90th centile). Physical exam revealed generalized hypotonia, hypersensitivity to sounds, hyperactivity, bilateral sandal gaps, strabismus and severe hyperopia (+8 diopters). She can speak only short sentences, but gives the impression that she understand everything. On the other hand, her behavior is somewhat autistic and she has problems with social interaction. She has short palpebral fissures, telecanthus, hypertelorism, flat and wide nasal root, bridge and tip, hypoplastic nares, low-set ears, micrognathia and high palate. Despite the facial dysmorphy she resembles her younger sister and her parents. Brain MRI examination at 5 years showed slightly dilated frontal horns and bodies of the lateral ventricles and very small anterior pituitary lobe. Posterior pituitary lobe were in normal position. There was no molar tooth sign or other abnormality of cerebellum or posterior fossa.

*ZNF423* mutations have been associated with Joubert syndrome/nephronophthisis, with autosomal dominant or recessive inheritance (MIM: [614844](https://omim.org/entry/614844))12-14. However, only 6 pathogenic *ZNF423* mutations have been reported, so the full spectrum of *ZNF423* associated symptomatology is probably not yet known. In particular, the patient described by Karaca et al., similar as our proband, did not have the molar tooth sign typical of Joubert syndrome13. Thus, at present it is not clear whether the clinical picture of our proband should be considered as further evidence that the *ZNF423* disease does not always include Joubert syndrome or the indication that her disease is caused by another mechanism than disruption of a single copy of *ZNF423*.

Proband 8 had a disruption of the *NIPBL* (MIM: 608667)gene linked to autosomal dominantly inherited Cornelia de Lange syndrome (MIM: [122470](https://omim.org/entry/122470))15, which was suspected in the proband prior to breakpoint mapping.

Disruption of *TRPS1* (MIM: 604386)was found in proband 9 - a male with dysmorphic features typical of Trichorhinophalangeal syndrome type 1 and 3 (MIM: [190350](https://omim.org/entry/190350), MIM: [190351](https://omim.org/entry/190351)) 16. The phenotype of the proband is well explained by monoallelic loss-of-function of *TRPS1* as such defects (including disruption by a translocation) are known to cause Trichorhinophalangeal syndrome I and II (MIM# 190350, 190351).

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