Left ventricular hypertrabeculation/noncompaction with epilepsy, other heart defects, minor facial anomalies and new copy number variants

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Abstract

Background: Left ventricular hypertrabeculation/noncompaction (LVHT) is a cardiac abnormality of unknown etiology which has been described in children as well as in adults with and without chromosomal aberrations. LVHT has been reported in association with various cardiac and extracardiac abnormalities like epilepsy and facial dysmorphism.

Case presentation: A unique combination of LVHT, atrial septal defect, pulmonary valve stenosis, aortic stenosis, epilepsy and minor facial anomalies is presented in a 5.5 years old girl. Microarray-based genomic hybridization (array-CGH) detected six previously not described copy number variants (CNVs) inherited from a clinically unaffected father and minimally affected mother, thus, most likely, not clinically significant but rare benign variants.

Conclusions: Despite this complex phenotype de novo microdeletions or microduplications were not detected by array CGH. Further investigations, such as whole exome sequencing, could reveal point mutations and small indels as the possible cause.

Keywords: Cardiomyopathy, Congenital heart disease, Neurology, Pediatrics, Array CGH, Hypertrabeculation, Seizures

Background

Left ventricular hypertrabeculation/noncompaction (LVHT) is a cardiac abnormality of unknown etiology which has been described in children as well as in adults. LVHT has been reported in association with various cardiac abnormalities, like Ebstein anomaly, pulmonary stenosis or atrial septal defect [1-12]. LVHT is associated with several extracardiac, especially neurological, abnormalities. LVHT has been reported in association with epilepsy, facial dysmorphism or minor anomalies [1,4,12-18]. Several chromosomal aberrations have been identified in LVHT associated with heart defects [1,4,8,10,12,15-17]. We present a pediatric patient with LVHT and other right and left heart defects, epilepsy and minor facial anomalies in whom microarray-based comparative genomic hybridization (array CGH) detected new copy number variants (CNVs).

Case presentation

A 5.5-year old girl had undergone surgical patch closure of a large secundum atrial septal defect with valvotomy of a moderate pulmonary stenosis at the age of 2 months; a mild aortic stenosis with a thickened tricuspid aortic valve was not corrected. She was born as the first child to non-consanguineous parents. The family history was negative for sudden cardiac death, but the maternal grandfather had epileptic seizures without fever in his youth. At the age of 12 months the girl suffered from a first afebrile seizure and at the age of 23 months from a first febrile seizure. Four months later she suffered from a series of afebrile seizures. Therefore valproic acid was started and maintained for 2 years. During this treatment she was

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seizure-free with normal electroencephalogram (EEG). After tapering valproic acid over a period of 2 months, short generalized paroxysmal discharges appeared on the EEG for the first time without clinical correlate. One year later she suffered from two further uncomplicated febrile seizures. Since then she is seizure-free without anticonvulsant treatment but the 24 h-EEG persistently shows subclinical absences. Since the intellectual and physical development of the patient is normal it was decided together with the parents not to restart the antiepileptic medication but to follow her up closely. Cerebral magnetic resonance imaging was normal. Clinical cardiologic, echocardiographic and neurologic examinations of the first degree relatives were normal except for the mother who showed epicanthic folds and hypertelorism. Unfortunately, the maternal grandfather did not consent with any investigations.

Figure 1 Frontal pictures of the patient showing bilateral epicanthic folds, broad eyebrows, broad nasal tip and hypertelorism.

Figure 2 Lateral pictures of the patient showing bilateral epicanthic folds, broad eyebrows, broad nasal tip and hypertelorism.

Figure 3 Echocardiographic apical 4-chamber-view showing the hypertrabeculated left ventricular apex with a two-layered structure.

Figure 4 Using colour-Doppler-sonography the intertrabecular recesses are perfused from the ventricular cavity.
At inspection, she had bilateral epicanthic folds; broad eyebrows, a broad nasal tip and hypertelorism, however no ptosis or low-set ears (Figures 1 and 2). Her body weight was 18 kg (25th percentile), head circumference 51 cm (50th percentile) and height 115 cm (75th percentile). She did not complain about any cardiac symptoms and did not suffer from heart failure. Twelve-lead electrocardiogram showed normal sinus rhythm alternating with an atrio-ventricular-nodal rhythm and an incomplete right-bundle-branch-block. Twenty-four-hour electrocardiogram showed sporadic ventricular and supraventricular ectopic beats. Echocardiography revealed a mild residual valvular pulmonary stenosis with moderate pulmonary valve regurgitation and mild aortic valve stenosis with mild aortic regurgitation. Left ventricular function was normal, however, extensive hypertrabeculation resulting in a two-layered structure of the myocardium was visible in the mid-ventricular and apical segments of the left ventricle, consistent with the diagnosis of LVHT (Figures 3 and 4). Review of previous echocardiographic examinations disclosed that LVHT had been present already at age 2 years. As a primary prophylaxis for cardiac embolism aspirin 50 mg/d was started. Since she was symptom-free, no further cardiac medication was prescribed. At the latest follow-up investigation in September 2011, she was in a good cardiac and neurologic condition, no seizures had recurred and her intellectual development was normal. Echocardiography was unchanged.

Molecular genetic analyses of the PTPN11, KRAS, RAF1 and SOS1 genes were negative. Array CGH using a 1 M oligonucleotide microarray platform (Agilent Technologies, Santa Clara, CA) was performed to screen genome-wide for submicroscopic deletions and duplications [19]. Genomic positions are given according to genome-build hg18. Array CGH detected five previously not described CNVs as listed in Table 1. FISH analyses were not carried out because of the size of the CNVs. Regarding the duplications it could not be assessed whether they were tandem-duplications on the same locus or whether the duplicated fragment was inserted or translocated in another chromosome. By application of quantitative Real-Time PCR (qPCR) we could show, however, that none of the changes had developed de novo. All five CNVs are currently not listed in the Toronto Database of Genomic Variants (DGV), therefore, have to be considered as non-frequent variants in normal controls. Follow-up and testing of the index patient and her parents by qPCR revealed that all CNVs were inherited from one of the unaffected parents (maternal: deletion 1q42.3, duplication 3q26.32q26.33; paternal: duplication 14q32.11, deletion and duplication 20q13.33). We thus conclude that these changes are most likely not clinically significant CNVs but rare benign variants, although a reduced penetrance of inherited CNVs cannot be excluded.

### Discussion
LVHT associated with atrial septal defect with or without pulmonary valve stenosis has been reported by several authors [2-9,11]. In most of these patients atrial septal defect and LVHT were associated with several other cardiac and extracardiac anomalies, and genetic studies revealed various mutations as listed in Table 2. However, the combination of a secundum type atrial septal defect with pulmonary stenosis and aortic stenosis in combination with LVHT has not been described to date. LVHT

| Chromosomal band | CNV | Start | End | Genes |
|------------------|-----|-------|-----|-------|
| 1p12             | del | 120338195 | 120404356 | NOTCH2 (intragenic, exon 2–4) |
| 1q42.3           | del | 234409418 | 234422919 | GPR137B (intragenic, exon 5) |
| 3q26.32q26.33    | dup | 180445165 | 180668097 | KCNMB3 (5' partial), ZNF639, MFN1, GNB4 |
| 14q32.11         | dup | 89942827 | 90019073 | CALM1 (3' partial) |
| 20q13.33         | del | 59412001 | 59460188 | CDH4 (intronic) |
| 20q13.33         | dup | 59597204 | 59629701 | CDH4 (intronic) |

Positions according to hg18.
associated with seizures has been reported in children with monosomy 1p36 [1,15,16] and interstitial 1q43 deletion [4]. Facial anomalies and LVHT have been described previously in children with developmental impairment, interstitial 1q43 deletion [4], deletion 1p36 syndrome [1], interstitial 8p23.1 deletion [12], point mutations and deletions of the NSD1 gene located at chromosome 5q35 [17] and in an adult with sex chromosome mosaicism, male phenotype and the karyotype mos45,X(28)/46,X,+mar(21)/47,X, + 2 mar(1) [18].

CNVs are structural genomic variants due to deletions or duplications, resulting in a copy-number change of the respective genomic region. CNVs may include entire genes, regions of transcribed sequence, or nontranscribed

Table 2 Reports about left ventricular noncompaction/hypertrabeculation with atrial septal defect

| Ref. | Age/sex | Additional cardiac findings | Extracardiocomorbidity | Follow-up | Genetic findings |
|------|---------|-----------------------------|------------------------|-----------|-----------------|
| [2]  | 39y/f   | Eb, heart failure, EF <55   | NI                     | Alive     | MTHF mutation   |
| [9]  | 16y/m   | Right pulmonary vein aplasia, left-sided pulmonary vein obstruction | NI                     | Alive     | NKO2.5 mutation |
| [10] | NI/m    | AV-block I, syncope        | NI                     | Alive     | NKX2.5 mutation |
| [11] | 23y/m   | VSD                         | NI                     | Alive     | NKX2.5 mutation |
| [3]  | Birth/f | VSD                         | Agenesis of corpus callosum, facial dysmorphism, febrile seizures | Alive at 3 years, delayed psychomotor development | Interstitial 1q43 deletion |
| [5]  | 16y/m   | None                        | NI                     | Alive     | E101K ACTC mutation |
| [5]  | 62y/m   | None                        | NI                     | Alive     | E101K ACTC mutation |
| [6]  | 1 m/m   | None                        | Hypotonia, developmental delay, nystagmus, strabismus, failure to thrive | Alive     | MMACHC mutation |
| [7]  | 5d/m    | EF <55, PDA, Eb             | NI                     | Alive     | NI              |
| [7]  | 1d/f    | None                        | NI                     | Alive     | NI              |
| [7]  | 6 m/m   | Ectopic atrial rhythm, VSD  | NI                     | Alive     | NI              |
| [7]  | 5y/f    | WPW, Eb                     | NI                     | Alive     | NI              |
| [7]  | 6 m/f   | EF <55, VSD, SAS, Coa       | NI                     | Dead      | NI              |
| [7]  | 2 m/m   | VSD                         | NI                     | Alive     | NI              |
| [7]  | 1 m/f   | SVT, VT, VSD, PDA, DILV    | NI                     | Alive     | NI              |
| [7]  | Birth/f | EF <55, PDA                 | Dead                   | NI        |                 |
| [7]  | 1d/f    | VSD, PDA, BAV, L SVC        | NI                     | Alive     | NI              |
| [7]  | 9 m/f   | EF <55, VSD                 | NI                     | Dead      | NI              |
| [7]  | 2d/m   | EF <55, VSD                 | NI                     | Dead      | NI              |
| [7]  | 3 m/m   | VSD                         | NI                     | Alive     | NI              |
| [7]  | 6d/m   | EF <55                       | NI                     | Alive     | NI              |
| [7]  | 9 m/m   | VSD                         | NI                     | Alive     | NI              |
| [7]  | 7d/f    | EF <55                       | NI                     | Alive     | NI              |
| [7]  | 1 m/m   | EF <55                       | NI                     | Alive     | NI              |
| [8]  | Birth/f | Sinusbradycardia, pulmonary valve atresia | BLI, ACV, abdominal situs ambiguous, polysplenia | Dead | Linkage to 5p24.3-21.2 |
| [8]  | 22y/m   | Sinusbradycardia, AF        | BLI, ACV                | Alive     | Linkage to 5p24.3-21.2 |
| [8]  | 32y/f   | Pulmonary valve stenosis, sick sinus syndrome | ACV, polysplenia, malrotation of the gut | Alive | Linkage to 5p24.3-21.2 |
| [8]  | 59y/m   | AF, heart failure, EF <55   | Polysplenia             | Died suddenly | Linkage to 5p24.3-21.2 |

NI, not indicated; EF, <55 % left ventricular ejection fraction; PDA, patent ductus arteriosus; Eb, Ebstein anomaly of the tricuspid valve; VSD, ventricular septal defect; WPW, Wolff-Parkinson-White; SAS, subaortic stenosis; Coa, coarctation of the aorta; SVT, supraventricular tachycardia; VT, ventricular tachycardia; DILV, double-inlet left ventricle; BAV, bicuspid aortic valve; L SVC, left-sided superior vena cava; ACV, azygous continuation of the vena cava inferior; AF, atrial fibrillation; BLI, bronchial left isomerism.
sequences. Whereas the duplication or deletion of a gene can be expected to have an effect on gene dosage, the consequences of CNVs in nontranscribed sequences are less clear [20]. According to the resolution of the applied array platform (5–10 kb genome-wide) we currently do not have evidence for a clinically relevant chromosomal imbalance i.e. gain or loss of genomic material. However, it is still possible that an underlying chromosomal abnormality either below the detection limit of the array or a balanced rearrangement which is undetectable by array CGH is causative for the described phenotype. This assumption is supported by the previously reported chromosomal defects in LVHT associated with facial anomalies, atrial septal defect and epilepsy. The presence of a chromosomal abnormality as the underlying defect is further substantiated by the fact that LVHT is particularly prevalent in young patients with chromosomal abnormalities [21].

In patients with epilepsy, an association with a truncation mutation of the KCNMB3 gene has been described which is partly affected from the duplication on chromosome 3 [22]. However, consequences of the duplication on the transcripts of this gene were not tested, and this is why such a presumed association remains speculative.

The first CNV detected in our patient (1p12 deletion) results in a deletion of part of the NOTCH2 gene that can be mutated in Alagille syndrome, a multisystem disorder with predominantly liver-, skeletal, ophthalmologic and renal abnormalities [23]. We consider Alagille syndrome unlikely in our patient since she did not show any of these abnormalities, although highly variable expressivity of the affected systems has been described in subjects with NOTCH2 mutations [24].

Conclusions
This case shows that childhood LVHT may be associated with other cardiac abnormalities, central nervous system disease and minor facial anomalies. Despite this complex phenotype de novo microdeletions or microduplications were not detected by array CGH. Further investigations, such as whole exome sequencing, could reveal point mutations and small indels as the possible cause.

Consent
The father of the patient has given his consent for the case report to be published.

Methods
Microarray-based comparative genomic hybridization (Array CGH)
Array CGH was performed using the 1 M oligonucleotide array (SurePrint G3 Human CGH 1x 1 M microarray, Agilent Technologies, Santa Clara, CA). We used female reference DNA (Human Genomic DNA female, Promega). Processing of the array was done according to the manufacturer's instructions. Extraction of microarray TIFF images had been done by “Feature Extraction” and the following data analysis was done by the “DNA Analytics 4.0” software (both Agilent Technologies, Santa Clara, CA). The following analysis settings in DNA analytics software were applied: algorithm ADM-2, filter 5 probes, log2ratio 0.29.

Quantitative real-time PCR (qPCR)
Genomic DNA samples of the index patient and her parents were obtained from EDTA-blood. Amplicons were located within the aberrant regions as detected by array CGH and in flanking regions. Primer sequences can be obtained upon request. qPCR was performed as previously described [20].

Abbreviations
Array CGH: Microarray-based comparative genomic hybridization; CNVs: Copy number variants; LVHT: Left ventricular hypertrobackulation/noncompaction.

Competing interest
The authors declare that they have no competing interests.

Authors’ contributions
BN collected clinical data, performed follow-up investigations, drafted the manuscript. UG-S collected clinical data, performed follow-up investigations, drafted the manuscript. SU performed the description of the dysmorphic features and took the photographs. CS drafted the manuscript, performed literature research, corresponding author. EK carried out the molecular genetic studies and drafted the manuscript. IF drafted the manuscript and performed literature research. All authors read and approved the final manuscript.

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