Clinical Case Reports

CASE REPORT

Prenatal homozygosity mapping detects a novel mutation in CHST3 in a fetus with skeletal dysplasia and joint dislocations

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Key Clinical Message

In selected cases, homozygosity mapping followed by direct sequencing of one or a few carefully selected candidate genes in a prenatal setting can be beneficial to obtain diagnosis in consanguineous families.

Case Report

In 1987, Lander et al. published the first paper on homozygosity mapping for human genes that cause recessive traits. He describes the usefulness of the method to map recessive diseases for which it is impractical or impossible to collect adequate numbers of families with multiple affected offspring [1]. Single nucleotide polymorphism (SNP) microarray has been documented to have clinical utility in the detection of regions of homozygosity [2]. In recent years, homozygosity mapping has demonstrated its effectiveness in the detection of disease loci in affected children from consanguineous couples in the neonatal setting, as well as after pregnancy termination for prenatally diagnosed malformations [3–6]. To our knowledge, the use of homozygosity mapping has only been reported once in the prenatal setting (on DNA isolated from amniotic fluid or a chorion villi biopsy) [7], although prenatal homozygosity mapping shows additional value because it can help parents to reach an underpinned decision on whether or not to terminate the pregnancy.

The case presented here involved a G3P2 woman, originating from Afghanistan, at 17 weeks gestational age. Her first two pregnancies were uncomplicated and resulted in two healthy boys. The couple was consanguineous (first cousins). In the current pregnancy, the gynecologist referred the couple for a suspicion of fetal limb malformation on routine ultrasound. Advanced ultrasound showed an abnormal morphology of the fetal lower limbs, short femora and humeri, abnormal position of the knee joints, and bilateral pes equinovarus, suggestive of arthrogryposis (Fig. 1). Family history showed that the brother of the proband's mother had had clinical arthrogryposis and had died during pregnancy (Fig. 2). Based upon the combination of ultrasonographic anomalies, differential diagnosis included: autosomal dominant Larsen syndrome, caused by mutations in *FLNB* [8], autosomal recessive Larsen syndrome, caused by mutations in *B3GAT3* [9], diastrophic dysplasia, caused by homozygous or compound heterozygous mutations in *SLC26A2* [10], Desbuquois dysplasia, caused by homozygous or compound heterozygous mutations in *CANT1* [11],

Figure 1. Advanced prenatal ultrasound showed an abnormal morphology of the fetal lower limbs. This figure shows the abnormal position of the knee joints and bilateral pes equinovarus on 3D ultrasound examination.

chondrodysplasia with joint dislocations, caused by homozygous mutations in *IMPAD1* [12], and spondyloepiphyseal dysplasia with congenital joint dislocations, caused by homozygous or compound heterozygous mutations in *CHST3* [13].

Given the anomalies found on prenatal ultrasound, amniocentesis was performed. Rapid aneuploidy testing (QF-PCR) did not reveal any aneuploidies. SNP array analysis to detect deletions and duplications was performed using a HumanCyto-SNP-12 v2.1 BeadChip on

an iScan system following standard protocols as provided by the manufacturer (Illumina, San Diego, CA, USA). Copy number variant (CNV) analysis was performed with CNV-WebStore [14]. SNP array analysis showed no pathogenic CNVs.

The pregnancy was terminated 2 weeks after the ultrasonographic findings because of the severity of the phenotype and after genetic counseling regarding future prospects for the unborn child. Postpartum clinical inspection of the fetus confirmed the joint and feet anomalies (Fig. 3). Radiographic examination confirmed the abnormal position of the left and right knee joints (Fig. 4). The ribs, upper limbs, and skull were normal. Autopsy report described a male fetus with biometry in the lower normal range for 17 weeks gestational age. Elbows showed a fixed flexion and knees had a fixed forward flexion as well. Macroscopically, there were no other remarkable skeletal anomalies. Facial features were normal. All organs in neck, thorax, abdomen, and pelvis were histologically and anatomically normal.

As the parents are first cousins, autozygosity, the presence of two alleles at a locus originating from a common ancestor as a result of inbreeding (identical by descent), might explain the phenotype; the fetus could have inherited the same mutant allele responsible for an autosomal recessive trait from both parents. To detect regions of autozygosity, possibly harboring a mutation, homozygosity mapping was performed on the sample obtained by amniocentesis using CNV-WebStore for data analysis [14]. Twenty seven homozygous regions larger than 1 Mb were identified, accounting for 8.43% of the genome (Table 1). Among the 2507 genes present in these regions,
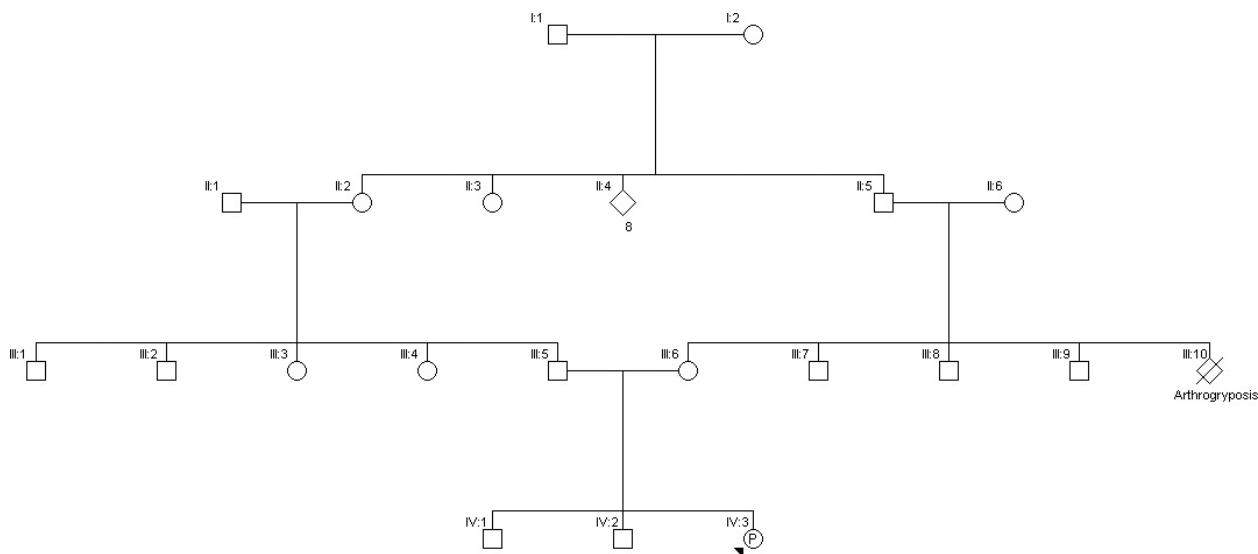

Figure 2. Family history. The unborn child is indicated as the proband (P-IV:3). Note the consanguinity of the parents (III:5 and III:6) (first cousins). Family history shows that the brother of the proband's mother (III:10) had had clinical arthrogryposis and had died during pregnancy.

Figure 3. Postnatal clinical inspection and autopsy confirmed the joint and feet anomalies.
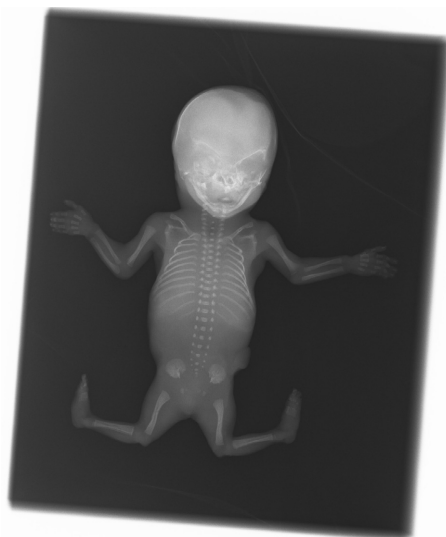

Figure 4. Radiographic examination confirmed the abnormal position of the left and right knee joints.

we carefully inspected all 362 MORBID genes. *CHST3*, located on chromosome 10q22.1, was considered to be the strongest candidate gene as homozygous and compound heterozygous mutations in this gene have been identified in spondyloepiphyseal dysplasia with congenital joint dislocations (OMIM #143095). This disorder is characterized by short stature (both prenatally and postnatally), a broad forehead, long philtrum, small ears, hypertelorism, high-arched palate, variable cardiac anomalies, broad chest, joint dislocations in knee, hip and shoulder, limb malformations (e.g., fixed elbow flexion and knee dislocations), brachydactyly, camptodactyly, and feet malformations. Affected individuals have a normal intelligence, but a delayed gross motor development. Our case presented with several characteristics representative for this disorder. DNA of the fetus and his parents was analyzed via PCR amplification and direct sequencing of *CHST3*. A hitherto unknown variant, c.491C>T (p.P164L), in exon 3 of the *CHST3* gene was detected in a homozygous state in the fetus. The proband's mother and father carry the mutation in a heterozygous state. This variant was classified as a likely pathogenic variant based on *in silico* predictions by Alamut (Alamut Visual version 2.7 [Interactive Biosoftware, Rouen, France]). This variant lies in the so-called P-loop containing nucleoside triphosphate hydrolase fold (amino acids 132–168). The Exome Aggregation Consortium (ExAc) [15] reports the heterozygous change of the same amino acid to Arg (Pro164Arg; rs771866012) in one of their 60700 individuals. The variant is classified as probably damaging by several *in silico* prediction programs, but no further studies have been performed. Mutations in three other amino acids in this domain have been described [16, 17]. Functional analysis of one of these, T141M, shows it to have a dramatically decreased sulfotransferase activity [16].

The proband's parents returned for further discussion 8 weeks after delivery. They received extensive genetic counseling and were informed about the possibility of preimplantation genetic diagnosis (PGD) or invasive testing in future pregnancies. The importance of further family testing was stressed. An informed consent for publication of the case was obtained after carefully informing the parents about the clinical significance of the case.

Since 2013, genomewide array – SNP array or array CGH (comparative genomic hybridization) – has replaced karyotyping in the prenatal setting in Belgium. At the time of introduction of this novel technology in the prenatal field, all Belgian genetic centers agreed on a resolution of 400 kb to maximize the detection of pathogenic CNVs while minimizing the detection of variants of unknown significance (VOUS) and on a list of susceptibility loci to report [18]. The increase in resolution from 5 to 10 Mb to 400 kb has led to an average increase of 6.0% of diagnoses in fetuses with ultrasound anomalies [19], but smaller deletions/duplications and single point

Table 1. Regions of homozygosity in the fetus.

Chromosome	Start	End	Size (bp)	Nr. Probes	% Hom	% Het	Nr. Genes
1	213827795	216840371	3012577	407	0.995	0.002	9
2	86023398	87052934	1029537	163	0.994	0.006	17
2	206714121	221239894	14525774	1017	0.999	0.001	136
3	96162805	97346618	1183814	151	1.000	0.000	1
4	31661814	40255501	8593688	553	0.996	0.004	32
4	75771799	157116213	81344415	5134	1.000	0.000	362
5	171324289	175220428	3896140	603	0.998	0.002	27
8	47921405	49039681	1118277	141	0.986	0.014	6
8	140733440	142600093	1866654	290	1.000	0.000	10
9	101297144	102540372	1243229	199	0.995	0.005	8
10	3239252	19471726	16232475	2045	1.000	0.000	99
10	37608337	38685231	1076895	137	1.000	0.000	10
10	71056150	95747476	24691327	2845	1.000	0.000	201
10	121922699	135430043	13507345	1950	0.999	0.001	105
11	2348778	11644920	9296143	1277	0.998	0.001	190
11	46196053	47246397	1050345	151	0.987	0.013	22
12	87738065	88801020	1062956	159	1.000	0.000	5
12	111768973	113025901	1256929	170	0.982	0.018	17
12	120822453	121874019	1051567	160	0.981	0.019	25
16	30172627	31383304	1210678	241	1.000	0.000	67
16	82678897	87111021	4432125	697	0.997	0.003	43
17	7314216	18747176	11432961	1698	0.999	0.000	184
17	37265378	75271787	38006410	3837	0.999	0.001	650
17	77291311	81047565	3756255	555	0.998	0.002	94
18	18540853	19601717	1060865	179	0.989	0.011	9
19	9247389	13355633	4108245	565	0.995	0.004	149
22	40731134	42196467	1465334	208	0.995	0.005	29

Indicated in the table are number of the chromosome; start and stop position of the region of homozygosity; size of the region in base pairs (bp); the number of probes in this region; percentage of homozygosity (% Hom) and heterozygosity (% Het) as well as the number of genes involved, accounting in total for 8.43% of the genome. Genome Build: GRCh37 (hg19).

mutations stay well below the radar. The arrival of high-density SNP array however [20, 21], as utilized in our center, allows the simultaneous determination of regions of homozygosity, which can lead to the detection of autosomal recessive mutations in offspring of consanguineous partners.

We could only identify one publication on the use of homozygosity mapping in the prenatal setting [7], making this report the second in its kind. The advantage of prenatal homozygosity mapping lies mainly in the short-term interval between recognition of the problem (ultrasound anomalies) and the definite diagnosis (a recessive mutation). In several countries, termination of pregnancy can only be legally performed until 24 weeks gestation, as the fetus becomes viable around that age. With the work-flow described here, complete results can be obtained within approximately 6 weeks: if amniocentesis is performed at 15 weeks gestational age, SNP array and homozygosity mapping results are known at 17 weeks gestational age and targeted analysis results at 21 weeks gestational age, well before 24 weeks. The result will guide gynecologists in counseling the parents correctly and can aid parents in

reaching a well-informed decision on whether or not to terminate the pregnancy. Moreover, as the parents can be screened immediately for carrier state of the recessive trait, they know whether or not they are at risk of having an affected fetus in a future pregnancy and can be counseled for the different options to avoid this, including PGD and invasive prenatal testing.

In this particular case, with the parents being first cousins, on average 6.25% of the genome is expected to be autozygous. Consequently, hundreds to thousands of putative candidate genes could be identified and prioritization of the genes, based on information regarding their function, is crucial. If the phenotype of the fetus can be clearly linked to one or a few of the genes, Sanger sequencing is the preferred method of choice. However, this approach precludes the identification of less obvious candidate genes. Therefore, in most cases successful autozygosity mapping relies on factors that further narrow the number of regions of interest like the investigation of multiple affected siblings. When no other affected family members are available, a combination of autozygosity mapping and next-generation sequencing (NGS) is

recommended, as it allows either massive parallel sequencing of all autozygous regions or filtering of variants found by whole-exome or whole-genome sequencing based on autozygosity mapping [22]. One can argue that autozygosity mapping can be performed directly on the NGS data, but cost and technical limitations of NGS (e.g., low read depth in some positions) preclude this for the time being. Moreover, if, based on the examination of the autozygome, a strong candidate gene is identified and sequenced, as was the case here, this is much more cost-effective than performing whole exome/whole-genome sequencing.

We conclude that in selected cases, homozygosity mapping followed by direct sequencing of one or a few carefully selected candidate genes in a prenatal setting can be beneficial to obtain diagnosis in consanguineous families.

Ethics

This manuscript is a case report and therefore did not require approval from our institution's ethics committee.

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Conflict of Interest

None declared.

Authorship

JM: main author of the paper, wrote the paper in close cooperation with KJ and gathered all information on the case and all reviewer's comments. BB: is the treating geneticist. She provided clinical insight in the case and reviewed the paper. YJ: is a gynecologist with expertise in prenatal diagnosis, provided clinical insight in the case, and reviewed the paper. KJ: is a main author of the paper and is the head of laboratory for prenatal genetics of the University of Antwerp.

References

1. Lander, E. S., and D. Botstein. 1987. Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. Science 236:1567–1570.

2. Sund, K. L., S. L. Zimmerman, C. Thomas, A. L. Mitchell, C. E. Prada, L. Grote, et al. 2013. Regions of homozygosity identified by SNP microarray analysis aid in the diagnosis of autosomal recessive disease and incidentally detect parental blood relationships. Genet. Med. 15:70–78.

3. Capo-Chichi, J. M., S. Boissel, E. Brustein, S. Pickles, C. Fallet-Bianco, C. Nassif, et al. 2015. Disruption of CLPB is associated with congenital microcephaly, severe encephalopathy and 3-methylglutaconic aciduria. J. Med. Genet. 52:303–311.

4. Xin, B., E. Puffenberger, J. Tumbush, J. R. Bockoven and H. Wang. 2007. Homozygosity for a novel splice site mutation in the cardiac myosin-binding protein C gene causes severe neonatal hypertrophic cardiomyopathy. Am. J. Med. Genet. A 143A:2662–2667.

5. Marshall, C. R., S. A. Farrell, D. Cushing, T. Paton, T. L. Stockley, D. J. Stavropoulos, et al. 2015. Whole-exome analysis of foetal autopsy tissue reveals a frameshift mutation in OBSL1, consistent with a diagnosis fo 3-M Syndrome. BMC Genom. 15(Suppl. 1):S12.

6. Fiskerstrand, T., G. Houge, S. Sund, D. Scheie, S. Leh, H. Boman, et al. 2010. Identification of a gene for renal-hepatic-pancreatic dysplasia by microarray-based homozygosity mapping. J. Mol. Diagn. 12:125–131.

7. Abumansour, I. S., E. Al Sulmi, B. N. Chodirker and J. C. Hunt. 2015. Prenatal diagnosis of walker-warburg syndrome using single nucleotide polymorphism array: a clinical experience from three related palestinian families with congenital hydrocephalus. AJP Rep. 5:e116–e120.

8. Krakow, D., S. Robertson, L. King, T. Morgan, E. T. Sebald, C. Bertolotto, et al. 2004. Mutations in the gene encoding filamin B disrupt vertebral segmentation, joint formation and skeletogenesis. Nat. Genet. 36:405–410.

9. Baasanjav, S., L. Al-Gazali, T. Hashiguchi, S. Mizumoto, B. Fischer, D. Horn, et al. 2011. Faulty initiation of proteoglycan synthesis causes cardiac and joint defects. Am. J. Hum. Genet. 89:15–27.

10. Hästbacka, J., A. de la Chapelle, M. Mahtani, G. Clines, M.P. Reeve-Daly, M. Daly, et al. 1994. The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78:1073–1087.

11. Faden, M., F. Al-Zahrani, D. Arafah, F. S. Alkuraya. 2010. Mutation of CANT1 causes Desbuquois dysplasia. Am. J. Med. Genet. 152A:1157–1160.

12. Vissers, L., E. Lausch, S. Unger, A. B. Campos-Xavier, C. Gilissen, A. Rossi, et al. 2011. Chondrodysplasia and abnormal joint development associated with mutations in IMPAD1, encoding the Golgi-resident nucleotide phosphatase, gPAPP. Am. J. Hum. Genet. 88:608–615.

13. Rajab, A., J. Kunze, and S. Mundlos. 2014. Spondyloepiphyseal dysplasia Omani type: a new recessive type of SED with progressive spinal involvement. Am. J. Med. Genet. 126A:413–419.

14. Vandeweyer, G., E. Reyniers, W. Wuyts, L. Rooms and R. F. Kooy. 2011. CNV-WebStore: online CNV analysis, storage and interpretation. BMC Bioinformatics 12:4.

15. Lek, M., K. J. Karczewski, E. V. Minikel, K. E. Samocha, E. Banks, T. Fennell, et al. 2016. The Exome Aggregation Consortium: Analysis of protein-coding variation in 60706 humans. Nature. 536(7616):285–91.

16. Tuysuz, B., S. Mizumoto, K. Sugahara, A. Celebi, S. Mundlos and S. Turkmen. 2009. Omani-type spondyloepiphyseal dysplasia with cardiac involvement caused by a missense mutation in CHST3. Clin. Genet. 75:375–383.

17. Unger, S., E. Lausch, A. Rossi, A. Mégarbané, D. Sillence, M. Alcausin, et al. 2010. Phenotypic features of carbohydrate sulfotransferase 3 (CHST3) deficiency in 24 patients: congenital dislocations and vertebral changes as principal diagnostic features. Am. J. Med. Genet. A 152A:2543–2549.

18. Vanakker, O., C. Vilain., K. Janssens, N. Van der Aa, G. Smits, C. Bandelier, *et al.* 2014. Implementation of genomic arrays in prenatal diagnosis: the Belgian

approach to meet the challenges. Eur. J. Med. Genet. 57:151–156.

19. Wapner, R. J., C. L. Martin, B. Levy, B. C. Ballif, C. M. Eng, J. M. Zachary, et al. 2012. Chromosomal microarray versus karyotyping for prenatal diagnosis. N. Engl. J. Med. 367:2175–2184.

20. Liao, C., F. Fu, R. Li, G. E. Xie, Y. L. Zhang, J. Li, et al. 2014. Implementation of high-resolution SNP arrays in the investigation of fetuses with ultrasound malformations: 5 years of clinical experience. Clin. Genet. 86:264–269.

21. Srebniak, M. I., M. Boter, G. O. Oudesluijs, T. Cohen-Overbeek, L. C. Govaerts, K. E. Diderich, et al. 2012. Genomic SNP array as a gold standard for prenatal diagnosis of foetal ultrasound abnormalities. Mol. Cytogenet. 5:14.

22. Alkuraya, F. S. 2013. The application of next-generation sequencing in the autozygosity mapping of human recessive diseases. Hum. Genet. 132:1197–1211.