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Variable levels of tissue mosaicism can confound the interpretation of chromosomal microarray results from peripheral blood

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Abstract

Chromosomal microarray analysis (CMA) has significantly increased the ability to diagnose medical conditions caused by copy-number variation in the human genome. Given that the regions involved in copy-number abnormalities often encompass multiple genes, it has been common practice in recent years to compare the phenotypes of individuals with specific copy-number alterations identified by CMA, with the goal of identifying the critical regions for particular elements of a disease phenotype. It is rarely mentioned that this practice relies heavily on the assumption that the absence of mosaicism on CMA from a peripheral blood sample (the most common source of DNA in current clinical practice) reflects the absence of mosaicism in other tissues. We report here a case that violates that assumption. A 28-year-old male with Charcot–Marie–Tooth disease was found by CMA to have a duplication of 17p12 along with two other abnormalities: A duplication of 12p13.33 translocated to the long arm of chromosome 3 and an interstitial duplication of 12p11.23. The patient did not have any clinical features suggestive of 12p duplication syndrome. Chromosomal microarray analysis on skin fibroblasts revealed the duplications at 17p12 and 12p11.23, but not the terminal duplication of 12p13.33. FISH analysis on skin fibroblasts confirmed the presence of very low levels of mosaicism for the terminal 12p duplication. The case illustrates how the absence of mosaicism in blood is not always indicative of the absence of mosaicism in other tissues. Even in an era of high-throughput, highly accurate DNA-based tests, it is important to remember the limitations of testing before drawing conclusions about the relationship between a test results and a clinical phenotype.

Keywords

Duplication 12p; Charcot–Marie–Tooth disease; Mosaicism; Array comparative genomic hybridization; Chromosomal abnormalities

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1. Clinical description

The patient was 28 years of age at the time of presentation. He is of Mexican ancestry. He was referred to the adult genetics clinic for evaluation of Charcot–Marie–Tooth Disease (CMT). His spouse was pregnant at the time of the initial visit and the couple was concerned about the risk of the fetus being affected with a similar condition. He reported a history of bilateral foot deformities that began at 11 years of age and progressively worsened. He had no personal history of intellectual disability, learning disorders, or seizure disorder. The patient was adopted and very limited information could be obtained about his family history, with the exception of unspecified foot deformities in a biological paternal aunt and cousin. Physical examination of the feet was remarkable for symmetric inward deviation, high arches, hammer-toe deformities, weak ankle dorsiflexion, atrophic distal muscles, depressed tendon reflexes, and distal sensory loss. There were no other dysmorphic features or abnormalities on physical examination. Nerve conduction studies performed at age 27 years showed markedly decreased motor conduction velocities, prolongation of distal motor latencies, and decreased amplitude in the right tibial, median and ulnar nerves. No sensory responses were recorded on the right sural nerve. Selective electromyography showed evidence of denervation and chronic neuropathic changes in the motor units, more marked in the lower extremities. Given the history, physical examination findings, and nerve conduction studies, a clinical diagnosis of Charcot-Marie-Tooth disease was made. Chromosomal microarray analysis was requested.

2. Methods

2.1. SNP microarray in peripheral blood

Whole genome SNP-based copy number microarray analysis targeting 2.695 million copy number and allele-specific genome sites was performed on a peripheral blood sample using the Affymetrix Cytoscan HD platform, which contains over 743,000 SNP reporters and 1,953,000 non-polymorphic copy-number reporters with a median spacing of 0.88 kb. Data was analyzed using Chromosome Analysis Suite. Nucleotide positions for the results of this microarray are based on the GRCh37/hg19 assembly.

2.2. Array-CGH in skin fibroblasts

A BCM Oligo V8.1 high-resolution microarray was employed for analysis of DNA from cultured skin fibroblasts. This array contains approximately 180,000 custom oligonucleotides and is manufactured by Agilent Technologies (Santa Clara, CA, USA). Average resolution across the genome is 30 Kb. 1714 genes have exon-by-exon coverage. The array also targets approximately 700 micro-RNAs and the entire mitochondrial genome. Nucleotide positions for the results of this microarray are based on the NCBI36/hg18 assembly.

2.3. Method of confirmation

Karyotype and FISH analyses were performed on peripheral blood lymphocytes using standard methods. FISH analysis was also performed on skin fibroblasts.

3. Genomic rearrangement

Chromosome analysis of GTG banded metaphases from a peripheral blood sample showed an unbalanced translocation of chromosomal material between the short arm of chromosome 12 and the long arm of chromosome 3 in all metaphases analyzed, suggesting obligate partial trisomy of 12p. SNP microarray on DNA from peripheral blood revealed a duplication in the short arm of chromosome 17:17p12(14,087,933–15,424,093)×3 (coordinates based on GCh37/hg19). It also showed two additional duplications, both involving the short arm of chromosome 12. One of these is a 6.8 Mb duplication at 12pter: 12p13.33p13.31(173,786–6,764,808)×3, which by FISH was found to be attached to the long arm of chromosome 3 without loss of material in 3. The duplicated region involves over 50 RefSeq genes. The other is a small (0.5 Mb) interstitial duplication: 12p11.23(27,293,774–27,793,755)×3. Array-CGH (oligonucleotide microarray) in skin fibroblasts identified the gains at 17p12 and 12p11.23, but did not reveal the terminal duplication of 12p13.33 (Fig. 1). Based on the result of this array, the 17p12 duplication encompasses a minimum interval of 1.294 Mb: 17p12(14,128,550–15,422,557)×3, and a maximum interval of 1.478 Mb: 17p12(14,078,559–15,551,757)×3 (coordinates based on NCBI36/hg18). The minimum interval for the 17p12 duplication includes the RefSeq genes *CDRT15*, *HS3ST3B1*, *MGC12916*, *CDRT7*, *PMP22*, *MIR4731*, *reg_PMP22_5*, *TEKT3*, *CDRT4*, and *TVP23C*. Also based on the result of the oligonucleotide array, the 12p11.23 duplication encompasses a minimum interval of 0.466 Mb: 12p11.23 (27,303,548–27,769,657)×3, and a maximum interval of 0.566 Mb: 12p11.23(27,232,361–27,797,928)×3. The minimum interval for the 12p11.23 duplication includes the RefSeq genes *STK38L*, *ARNTL2*, *SMCO2*, *PPFIBP1*, and *MRPS35*. To rule out low-level mosaicism for the terminal 12p duplication, FISH analysis on the skin fibroblast sample was performed. This again showed the 17p12 duplication (Fig. 2A) and excluded the possibility of an insertional translocation event for the small duplication at 12p11.23. FISH analysis on skin fibroblasts with chromosome 12 telomeric probes (12pter and 12qter) showed an additional signal of 12pter, at the end of the long arm of chromosome 3, in only one of 35 metaphase cells studied (Fig. 2B). Parental studies were not done as the patient is adopted and has no information about his biological parents.

4. Discussion

We present the case of a 28-year-old with a clinical and electrophysiological diagnosis of Charcot–Marie–Tooth (CMT) disease. A SNP microarray confirmed the diagnosis of CMT type 1a, by demonstrating the presence of a duplication at 17p12, encompassing *PMP22*. Two other duplications were also found in non-mosaic form in peripheral blood, both within the short arm of chromosome 12. Clinically, however, the patient did not have any phenotypic features of a genetic condition other than CMT type 1a. Duplication of 12p has been reported in individuals with facial dysmorphism, hypotonia, intellectual disability, seizures, and other congenital abnormalities [Segel et al., 2006]. Patients with mosaic 12p duplications appear to have better outcomes than those with complete 12p duplication or with duplication of 12p and other chromosomal anomalies [Allen et al., 1996]. The typical appearance associated with partial trisomy 12p was earlier reported to occur with duplication of 12pter alone [Stengel-Rutkowski et al., 1981], but the critical region has since

been suggested to correspond to 12p13.1p13.33, where the phenotype was reported to manifest even in mosaic state [Rauch et al., 1996]. More recently, the report of two members of one family with a duplication of the distal 6 Mb of 12p (corresponding to 12p13.31p13.33) and no discernible disease phenotype [Madrigal et al., 2012] suggests that the critical dosage-sensitive genes in the region may be proximal to 12p13.31. Duplications in this region are also observed as somatic events in peripheral blood cells in hematologic malignancies, including 5% of cases of acute non-lymphocytic leukemias (ANLL) and myelodysplastic syndromes (MDS), and 30% of cases of acute lymphoblastic leukemias.

With the exception of *MRPS35*, duplication of the genes at 12p11.23 that were observed to be duplicated in our patient has been described in control populations and is included in the Database of Genomic Variants (Variant: nsv898940). The DECIPHER database currently includes the case of an individual (Patient 251557) with a reported phenotype of short stature and brachydactyly, who was found to have a larger duplication of this region that also involves the genes *MANSC4*, *KLHL42*, *PTHLH*, and the 5' end of *CCDC91*. Based on this information, we felt that the presence of this duplication in our patient was consistent with the absence of a phenotype other than that of CMT type 1a.

On the other hand, the 6.8-Mb terminal 12p duplication that was identified in our patient presented a clinical dilemma, both in terms of interpretation of the test results and in terms of counseling for him and his pregnant spouse. The duplication was 800 Kb larger than that previously described in this region in individuals without a disease phenotype [Madrigal et al., 2012], and it included the previously suspected critical region for 12p duplication [Rauch et al., 1996]. Given the absence of clinical features of 12p duplication in our patient, we considered three hypotheses: (1) The patient could have the duplication in non-mosaic state, as suggested by the array-CGH results in peripheral blood, (2) the 12p duplication could be a somatic event in peripheral blood cells alone, or (3) the duplication could be non-mosaic in blood but mosaic in other tissues. We decided to test these hypotheses by obtaining a skin fibroblast sample and performing array-CGH. This showed the proximal 12p duplication, but not the terminal 12p duplication that was also seen in peripheral blood. Additional analysis by FISH on the skin fibroblasts did reveal the terminal 12p duplication, however at very low levels of mosaicism, suggesting a constitutional event rather than a post-natal somatic event in the bone marrow.

Array-CGH is most often performed in peripheral blood, and occasionally in saliva samples. In clinical practice, as well as in the ongoing large-scale efforts to associate specific chromosomal imbalances with human phenotypes, an assumption is often made that the absence of mosaicism in the tissue tested is indicative of an absence of mosaicism in all other tissues. Under that assumption, one could have used this case as additional evidence that duplications of the terminal end of 12p are not associated with a discernible clinical phenotype and could have attempted to further delineate the critical region of 12p duplications to exclude the distal 6.8 Mb, a region larger than the 6 Mb previously reported in two unaffected individuals [Madrigal et al., 2012]. Instead, this case provides a specific example of how that assumption is not always true. Even in an era of high-throughput, highly accurate DNA-based tests, it is important to remember the limitations of testing

before drawing conclusions about the relationship between a test results and a clinical phenotype.

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Fig. 1. Array-CGH (oligonucleotide array) results from the patient's fibroblasts. The green dots indicate copy number gains on chromosomes 17p12 and 12p11.23. The terminal 12p duplication that was documented in peripheral blood was not found in the patient's fibroblasts.

Fig. 2.

FISH images from the patient's skin fibroblasts: **(A)** Duplication of the CMT 1a region in 17p12, in interphase nuclei. **(B)** Metaphase spread showing duplication of 12pter, with the additional signal at the end of the long arm of chromosome 3, an event seen in only 1 of 35 metaphase fibroblasts studied.