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CLINICAL REPORT

Chromosomal translocation disrupting the *SMAD4* gene resulting in the combined phenotype of Juvenile polyposis syndrome and Hereditary Hemorrhagic Telangiectasia

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ABSTRACT

Background: Patients with germline variants in *SMAD4* can present symptoms of both juvenile polyposis syndrome (JPS) and Hereditary Hemorrhagic Telangiectasia (HHT): JP-HHT syndrome. Next-Generation Sequencing (NGS) techniques disclose causative sequence variants in around 90% of HHT patients fulfilling the Curaçao criteria. Here we report a translocation event involving *SMAD4* resulting in JP-HHT.

Methods: A patient fulfilling the Curaçao criteria was analyzed for variants in *ENG*, *ACVRL1*, and *SMAD4* using standard techniques. Whole-genome sequencing (WGS) using both short-read NGS technology and long-read Oxford Nanopore technology was performed to define the structural variant and exact breakpoints.

Results: No pathogenic variant was detected in *ENG*, *ACVRL1*, or *SMAD4* in DNA extracted from blood. Due to abortus habitualis, the proband´s daughter was submitted for chromosomal analysis, and a cytogenetically balanced chromosomal reciprocal translocation t(1;18)(p36.1;q21.1) was detected in the daughter and the patient. The balanced translocation segregated with both gastrointestinal cancer and HHT in the family. WGS provided the exact breakpoints of the reciprocal translocation proving disruption of the *SMAD4* gene.

Discussion: A disease-causing reciprocal translocation between chromosome 1 and 18 with a breakpoint in the *SMAD4* locus co-segregated with JP-HHT in an extended family. This observation warrants further analysis for chromosomal rearrangements in individuals with clinical HHT or JP-HHT of unknown cause.

KEYWORDS

balanced translocation, chromosomal translocation, gastrointestinal cancer, Hereditary Hemorrhagic Telangiectasia, HHT, JP-HHT, JPHT, juvenile polyposis syndrome, SMAD4

Abbreviations: ACVRL1, Activin A Receptor Receptor-Like Type 1; CNV, copy number variation; ENG, endoglin; GI, gastrointestinal; HHT, hereditary hemorrhagic telangiectasia; IGV, integrative Genomics Viewer; JP-HHT, juvenile polyposis - hereditary hemorrhagic telangiectasia; JPS, juvenile polyposis syndrome; NGS, generation sequencing techniques; PAVM, pulmonary arteriovenous malformations; SMAD4, mothers against decapentaplegic homolog 4; WGS, whole genome sequencing.

1 | INTRODUCTION

The autosomal dominant hereditary disorders Juvenile Polyposis Syndrome (JPS, OMIM 174900) and Hereditary Hemorrhagic Telangiectasia (HHT, OMIM 187300), also known as Osler-Weber-Rendu disease, are separately well-known and well-described conditions. Less known is the JP-HHT syndrome (OMIM 175050) and the phenotypic characteristics of this syndrome are still to be fully elaborated.

HHT presents with multiple mucocutaneous telangiectases and arteriovenous malformations (AVMs) in internal organs. The most prominent clinical manifestation is recurrent epistaxis which occurs in more than 90%. (Folz, Tennie, Lippert, & Werner, 2005). Another frequent symptom is gastrointestinal (GI) bleedings, which are detected in 33%. These symptoms are considered to increase by age. (Kjeldsen & Kjeldsen, 2000). Pulmonary arteriovenous malformations (PAVMs) are discovered in approximately one-third of HHT patients and require treatment to prevent severe complications (Govani & Shovlin, 2009; Kjeldsen, Oxhoj, Andersen, Green, & Vase, 2000; Kjeldsen, Torring, Nissen, & Andersen, 2014; Mathis et al., 2012; Shovlin et al., 2008). HHT is a clinical diagnosis and the patients are examined and diagnosed according to the Curaçao criteria (Shovlin et al., 2000).

A pathogenic variant in one of three genes are known to cause HHT: *ENG* (OMIM 131195, HHT1; McAllister et al., 1994), *ACVRL1* (OMIM 601284, HHT2; Johnson et al., 1995) and *SMAD4* (OMIM 600993, JP-HHT; Gallione et al., 2004). About 85% of the patients have a pathogenic variant in *ENG* or *ACVRL1* (Torring, Brusgaard, Ousager, Andersen, & Kjeldsen, 2014). In addition, two loci on chromosome 5q31 (Cole, Begbie, Wallace, & Shovlin, 2005) and 7p14 (Bayrak-Toydemir et al., 2006) have, years ago, been linked to HHT, but the relevant genes have not yet been mapped. Variants in *BMP9* have been suspected to cause a vascular-anomaly syndrome with phenotypic overlap with HHT (Wooderchak-Donahue et al., 2013), although this has not yet been confirmed (Tørring et al., 2016).

Patients with Juvenile Polyposis Syndrome (JPS) presents with multiple juvenile polyps in the alimentary canal. The most often detected symptoms are pain in the abdomen, anemia, rectal bleeding, and anal prolapsing polyps (Manfredi, 2010). The polyps can occur in all regions of the GI tract and vary from a few to more than a 100. The lifetime risk for GI cancer for these patients is increased (Brosens et al., 2007; Howe, Mitros, & Summers, 1998). When analyzing the patients who fulfill the defined diagnostic criteria for JPS (Jass, Williams, Bussey, & Morson, 1988), a germline pathogenic variant in *BMPR1A* or *SMAD4* can be identified in at least 60% (Aretz et al., 2007; Latchford, Neale, Phillips, & Clark, 2012).

In 2004, a combined syndrome consisting of both JPS and HHT phenotypes was described (Gallione et al., 2004). JP-HHT syndrome is caused by a germline pathogenic variant of the *SMAD4* gene, which is mapped to chromosome 18q21. Those variants are identified in 3-10% of HHT-patients, and these patients are at high risk of developing JP-HHT (Gallione et al., 2006; Jelsig et al., 2015; Karlsson & Cherif, 2018; Torring et al., 2014). A wide range of pathogenic variants of *SMAD4* has been reported (Gallione et al., 2010), the majority of genetic aberrations being unique in each family. To the best of our knowledge, the current case is the first report describing a chromosomal translocation event involving the *SMAD4* loci and resulting in the combined syndrome of JPS and HHT.

Furthermore, patients with JP-HHT are at risk of thoracic aortic dilatation (Jelsig et al., 2015). Multidisciplinary surveillance from childhood for carriers of *SMAD4* mutations is essential as complications to polyps, cancer, AV-malformations, and aortopathy, may be avoided or ameliorated.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

Informed consent was obtained from all family members included in this study.

2.2 | Patients

The pedigree of the family appears in Figure 1. The proband (III:8, Figure 1) underwent clinical examination for HHT. She was found to fulfill all four Curaçao criteria having epistaxis, telangiectatic lesions, PAVM and a first degree relative with HHT. Her son (IV:8, Figure 1) also fulfilled all four Curaçao criteria. The daughter (IV:7, Figure 1) fulfilled three of four Curaçao criteria, as no PAVMs were identified during contrast echocardiography. The same applied to the proband´s cousin (III:1, Figure 1), who only presented with few telangiectatic lesions and epistaxis at the time of examination. He had anemia, multiple polyps in the colon, and was diagnosed with c. coeci (colorectal cancer) twice, the first time at 33 years of age. The proband's father (II:3, Figure 1) was diagnosed with colorectal cancer and had symptoms of HHT. The proband's paternal aunt (II:2, Figure 1) was diagnosed with c. ventriculi and both her and the proband's paternal uncle (II:5, Figure 1) had symptoms of HHT. The remaining family members did not have a clinical examination regarding HHT manifestations, but was reported without any symptoms.

2.3 | HHT panel analysis

Samples were sequenced utilizing a targeted NGS HHT panel including *ENG* (NM_001114753.1), *ACVRL1* (NM_000020.2) and *SMAD4* (NM_005359.5) utilizing the Agilent targeted sequence capture method, followed by sequencing on an Illumina HiSeq1500 NGS platform (Illumina Inc). The target region included all exons, 5′- and 3′-UTRs, and 50 kb into the flanking introns. The SureSelectXT Reagent kit (Agilent Technologies Denmark ApS) was applied and libraries were run in a single lane using paired-end sequencing at 2 × 100 bp.

2.4 | Chromosome analysis

Standard procedures of chromosomal analysis were applied. In short, leucocytes were cultured with phytohaemagglutinin, interrupting the cell divisions by colcemid solution, Leishman´s color was used for staining the chromosomes (Figure 2). To achieve the best results, the analysis of at least 12 metaphases was performed.

2.5 | Whole-genome sequencing, short-read

In order to clarify previous findings and fine-map the translocation breakpoints, DNA was extracted and paired-end whole-genome sequencing (2 × 150 bp) was performed using a NovaSeq 6000 (Illumina) platform. Library preparation for sequencing was performed using the TruSeq PCR-free (Illumina) kit. The mean genome coverage achieved by NGS was 34.3X. Bioinformatics data analysis as well as a structural variant calling was performed using DRAGEN (Illumina) software. Integrative Genomics Viewer (IGV) software was used for data visualization. Breakpoint detection and fine-mapping was performed by the manual exploration of the region of interest on IGV (Figure 3).

2.6 | Whole-genome sequencing, long-read

To validate the breakpoints, long-read Oxford Nanopore sequencing was performed. HMW-DNA was extracted using Nanobind CBB Big DNA Kit (Circulomics). Sequencing library was prepared using Ligation Sequencing Kit (Oxford Nanopore Technologies) and sequenced on a PromethION (Oxford Nanopore Technology) using one R9.4.1 flow cell with a total yield of 64 Gbases (N50: 18.6 Kb). The mean genome coverage achieved was 17X. Alignment and structural variant calling was performed using minimap2 and sniffles software. Integrative Genomics Viewer (IGV) software was used for data visualization and fine-mapping of the breakpoint (Figure S1).

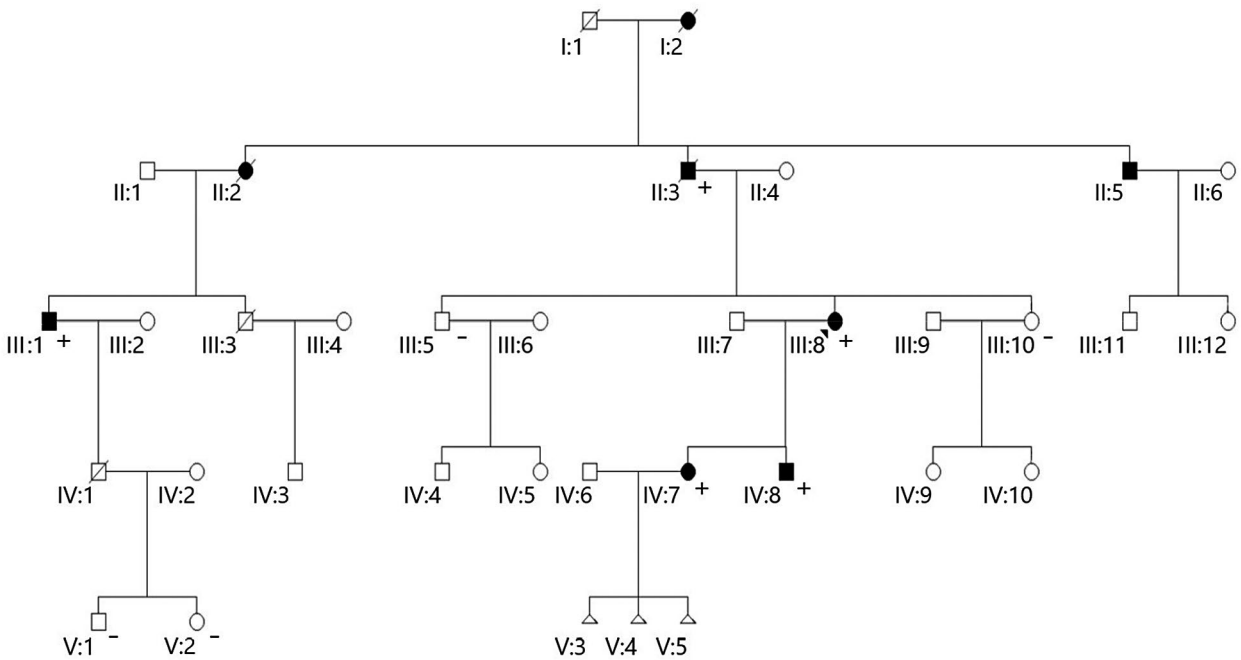

FIGURE 1 Family pedigree. Arrow marks the proband. —, not translocation carrier; +, translocation carrier; Black, affected with JP-HHT; White, non-affected.

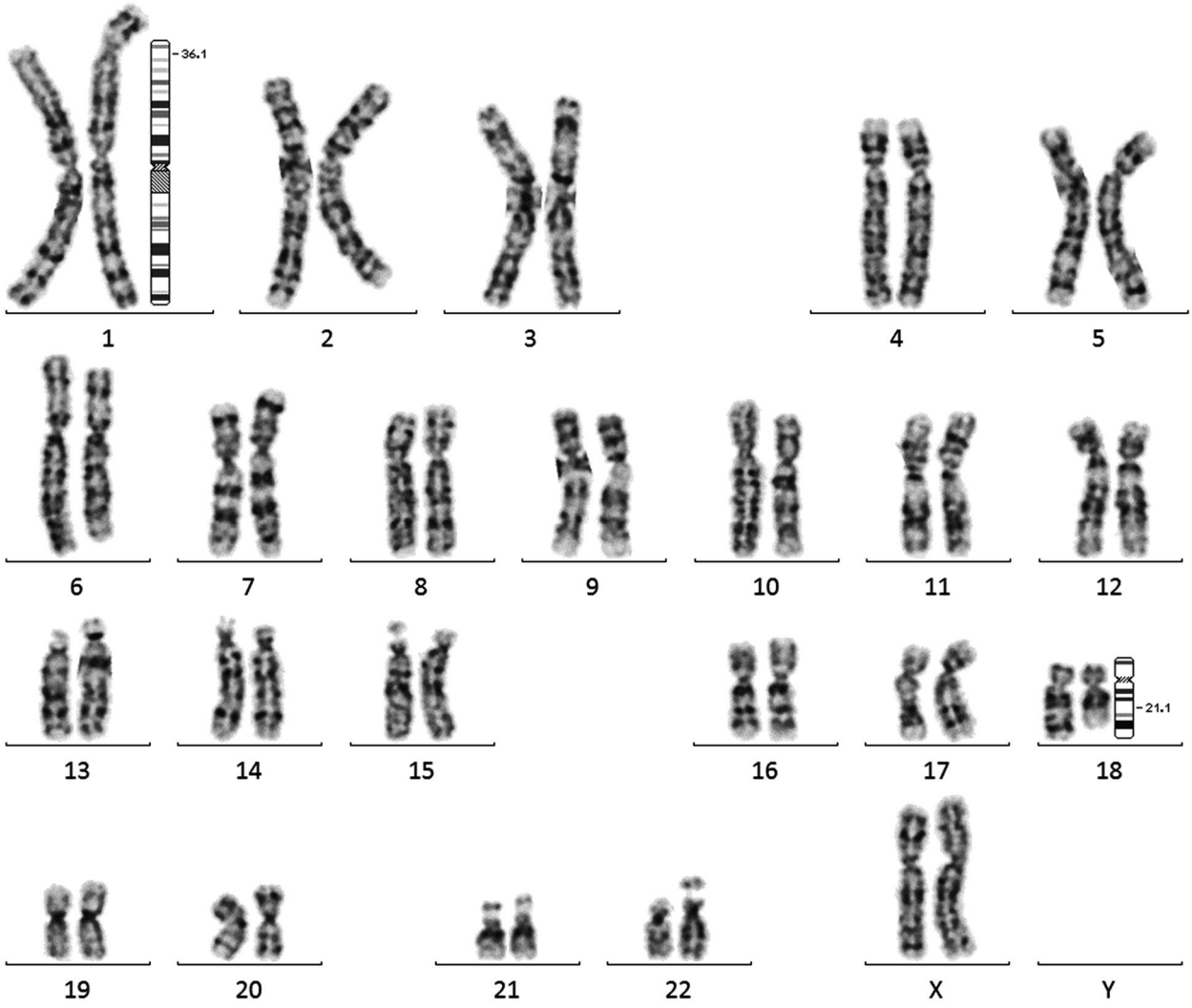

FIGURE 2 Chromosomal analysis of the proband's daughter with the balanced reciprocal translocation t(1;18)(p36.1;q21.1), which was subsequently found in the affected family members. A schematic chromosome 1 and 18 is inserted.

3 | RESULTS

Due to the clinical findings, the proband (III:8, Figure 1) was submitted to targeted HHT panel analysis. No pathogenic variants of neither *ENG*, *ACVRL1* or *SMAD4* were observed.

At a later stage, the proband's daughter (IV:7, Figure 1) underwent chromosomal analysis due to several spontaneous abortions. A cytogenetically balanced reciprocal translocation involving chromosomes 1 and 18 ((1;18)(p36.1;q21.1, Figure 2) was revealed.

Whole-genome sequencing data analysis called both breakpoints of the translocation. Manual exploration of the regions of interest refined the breakpoints and confirmed the cytogenetically balanced reciprocal translocation involving chromosome 1 and chromosome 18 with a breakpoint placed between exons 5 and 6 of the *SMAD4* gene, thus disrupting the gene (Figure 3 and Figure S1). According to ISCN guidelines, the structural variant was named as seq[GRCh37] t(1;18)(p36.1;q21.1) g.[chr18:48583533_qterinv::ACA::chr1:13774519_cen_qter] g.[chr18:pter_cen_48583541::chr1:13774520_pterinv]. It describes a translocation between the chromosomes 1 (short arm) and 18 (long arm), with an insertion of a non-templated 3 bp sequence at the breakpoint on the derivative chromosome 1. There is an overlapping sequence from chromosome 18 seen on both derivative chromosomes, which can be observed from the nucleotide numbers given for the two chromosome 18 breakpoints (Figure 3). To verify the breakpoints of the translocation, we performed long-read whole-genome sequencing using Nanopore technology. The results of the long-read WGS confirmed both breakpoints with high confidence (Figure S1).

Discovering the *SMAD4* variant, further clinical screening of the family revealed characteristic JPS symptoms in the form of multiple juvenile polyps in the son of the proband (IV:8, Figure 1) at the age of 35 years. Also, the proband's cousin (III:1, Figure 1), who prior to this finding had both multiple polyps and GI cancer, had once more several polyps

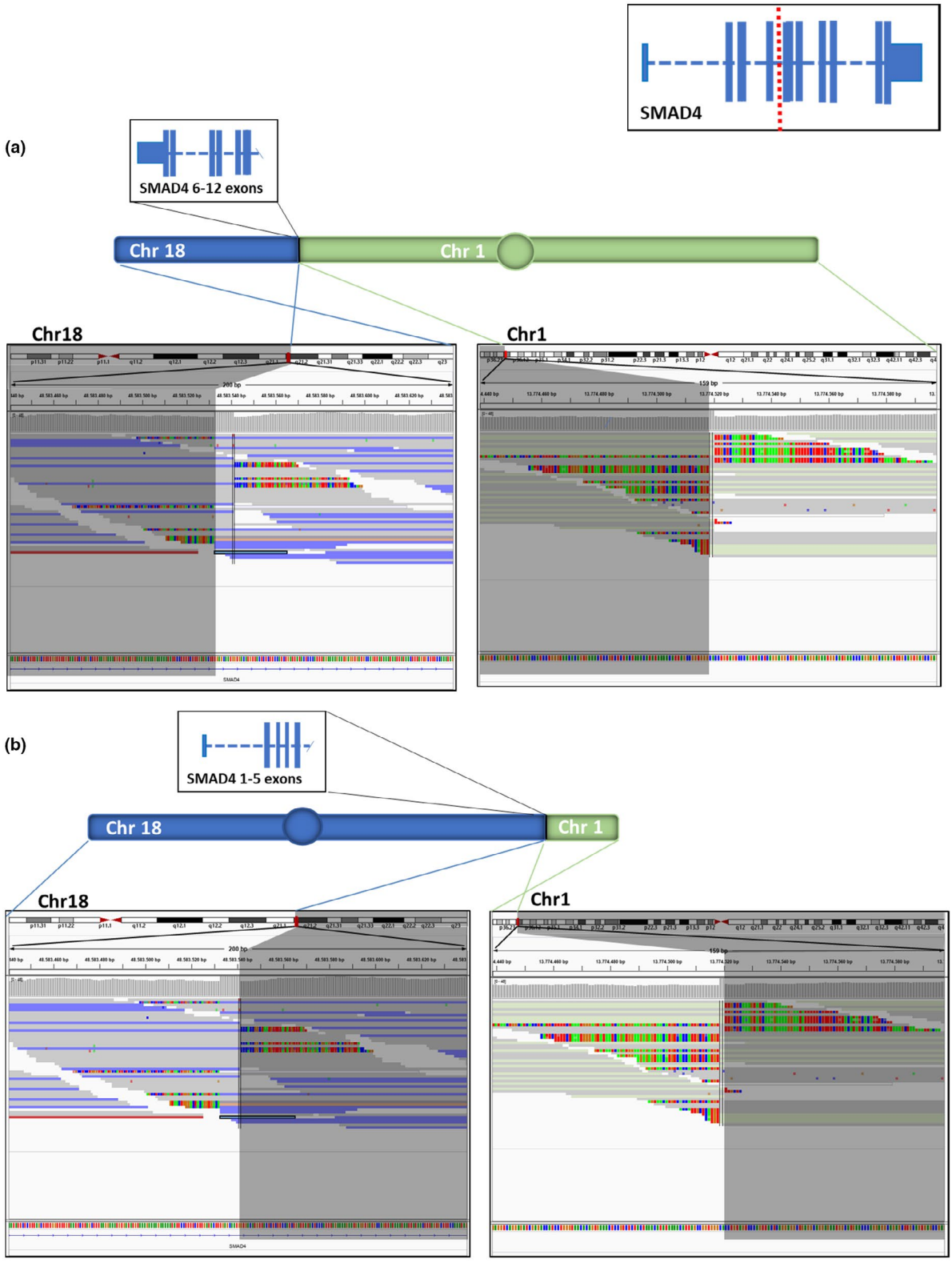

FIGURE 3 WGS results of a cytogenetically balanced translocation between chromosomes 1 and 18 with a breakpoint in the *SMAD4* gene identified in a patient with JP-HHT. The breakpoint in the *SMAD4* gene maps to the intron between exons 5 and 6 (shown in the top-right corner by the red dotted line). WGS data analysis results are visualized by IGV snapshots of the breakpoint (a and b). Rainbow-colored parts seen bellow refer to misaligned bases on the reads sequencing the breakpoint, that is, split reads. The split read after the breakpoint aligns with another chromosome. (a) Shows the derivative chromosome 1 involving seven (6-12) exons of the *SMAD4* gene. Genomic coordinates: g.[chr18:48583533_qterinv::ACA::chr1:13774519_cen_qter] with 3-bp (ACA) non-template sequence insertion at the breakpoint. The breakpoint on chromosome 1 is in the non-coding region, thus no gene is disrupted by it. (b) Shows the derivative chromosome 18 involving five (1-5) exons of the *SMAD4* gene. Genomic coordinates: g.[chr18:pter_cen_48583541::chr1:13774520_pterinv]. Both derivative chromosomes have 41 read pairs supporting the translocation and 12 split reads supporting the breakpoints.

removed which histologically was now confirmed as juvenile polyps. A single none-juvenile polyp was removed in the proband, and in the daughter 2 polyps, whereas one was atypically located in cardia, but unfortunately, this was not histologically examined.

Relevant family members were offered screening for thoracic aortopathy, which was not identified.

As a whole, the family presented with several overlapping cases of gastrointestinal cancer, colonic polyps, and HHT. In the currently alive phenotypically affected family members, the translocation has been identified.

4 | DISCUSSION

The current study presents the molecular genetic evaluation of a family with HHT and JPS. The conditions JPS and HHT, can both be caused by variants in the *SMAD4* gene and the view of this research group is that all patients with a pathogenic variant of *SMAD4*, should be suspected of JP-HHT. However, the HHT symptoms are known to be varying and can, therefore, be mild and the penetrance is age-dependent, for which reason the diagnosis is not always clinically confirmed or even suspected. Further, the JPS symptoms are also varying and age-dependent, which is clearly presented in this family. Nonetheless, most of the affected family members seem to have convincing symptoms of both HHT and JPS, which is in line with the other *SMAD4* families, in our Center, that are all offered screened for both HHT and JPS.

The proband of this study fulfilled the Curaçao criteria, but HHT-targeted panel analysis including *SMAD4*, did not disclose a pathogenic variant. The disease-causing aberration was discovered by chance due to chromosomal analysis on a separate indication. The exact breakpoints were subsequently determined, using both short-read (NGS) and long-read WGS, independently confirming the disruption of *SMAD4* (Figure 3 and Figure S1).

Standard gene panel methods will not reliably reveal structural chromosomal variants, in contrast to conventional karyotyping especially with FISH. However, chromosomal analysis has a low resolution and cannot identify the exact breakpoints. Therefore, WGS of relevant genes is the approach of choice, as both coding, non-coding and structural variants may be identified in the selected loci. In the current case, standard short-read WGS was sufficient to identify the breakpoint. However, chromosomal breakpoints often happen to occur in low-complexity regions of the genome (sequence that is either repetitive or highly homologous with other genomic regions, incl. pseudogenes) which make them difficult to detect by short-read sequencing. Due to the advantage of the sequencing of longer DNA fragments, long-read technologies such as Oxford Nanopore or PacBio sequencing methods is likely to be a much better choice to resolve complex structural chromosomal events (Logsdon, Vollger, & Eichler, 2020; Mitsuhashi & Matsumoto, 2020).

5 | CONCLUSION

To the extent of our knowledge, an incidence of a chromosomal translocation with the breakpoints in the *SMAD4* resulting in the combined JP-HHT syndrome has not been reported before, which indicates it to be relatively rare. The findings of this study point to that further analysis, in order to discover chromosomal rearrangements, should be taken into consideration when targeted sequencing does not reveal a molecular genetic cause of disease in individuals or families with HHT and JP-HHT. Conventional short-read WGS sequencing is the obvious choice for this purpose, but in this study, the clinical utility of long-read sequencing in rare disease diagnostics by characterizing a balanced reciprocal translocation is also demonstrated.

Patients with *SMAD4* mutations should be screened for both HHT, JPS, and aortopathy. Identifying the variant and thereby being able to reach a definitive and complete diagnosis, is of great importance for the affected family members in order to receive sufficient counseling and surveillance.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

Performed the clinical examinations: ADK, PMT. Performed the data analysis incl. interpretation of data: MJL, IM, KB, LBO, EBL, PMT. Acquisition of data: KSA, PMT, ADK. Drafted the first version of the manuscript: KSA, PMT. All authors have been involved in revising the manuscript and have given approval of the final version.

DATA AVAILABILITY STATEMENT

The data generated and analyzed as part of the current study are not publicly available due to personal data information on the patients. Access to data is restricted to the authors. Anonymized parts of the data can be retrieved from the corresponding author on a reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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