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CLINICAL REPORT

Identification of pathogenic *YY1AP1* splice variants in siblings with Grange syndrome by whole exome sequencing

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Grange syndrome is an autosomal recessive condition characterized by arterial occlusions and hypertension. Syndactyly, brachydactyly, bone fragility, heart defects, and learning disabilities have also been reported. Loss-of-function variants in *YY1AP1* have only recently been associated with Grange syndrome. *YY1AP1* encodes for the transcription coactivator yin yang 1-associated protein 1 which regulates smooth muscle cell proliferation and differentiation. We here report on three siblings with steno-occlusive arterial disorder and syndactyly in two of them. Whole exome sequencing including near-splice regions led to the identification of two intronic *YY1AP1* variants which were predicted to interfere with normal splicing. Sanger sequencing demonstrated compound-heterozygosity in all affected siblings. RT-PCR analyses confirmed skipping of exon 6 on one allele and exonization of 22 bp in intron 6 on the other. This is the first report of biallelic *YY1AP1* variants in noncoding regions and just the second family with multiple affected siblings. Therefore, our report further delineates the phenotypic spectrum of Grange syndrome.

KEYWORDS

Grange syndrome, splice variant, vascular disease, whole exome sequencing

1 | INTRODUCTION

Grange syndrome (OMIM: #602531) is a rare, early-onset disease characterized by hypertension and multifocal steno-occlusive lesions of renal, cerebral and abdominal arteries. It was first described in 1998 in a family with four affected siblings (Grange, Balfour, Chen, & Wood, 1998). Bone fragility, syndactyly, brachydactyly, congenital heart defects, and learning disabilities appear to be associated with variable expressivity and incomplete penetrance (Grange et al., 1998; Volonghi et al., 2012; Wallerstein et al., 2006; Weymann et al., 2001). The genetic etiology of Grange syndrome has only recently been clarified. Guo et al. (2017) identified five distinct homozygous and compound-heterozygous loss-of-function variants in the *YY1AP1* gene of six affected probands. *YY1AP1* encodes for the widely expressed transcription coactivator yin yang 1-associated protein 1. YY1AP1 and its partner YY1 are components of the INO80 chromatin remodeling complex and act as regulators of proliferation and differentiation in aortic smooth muscle cells (Guo et al., 2017).

The vascular phenotype of Grange syndrome resembles some features of fibromuscular dysplasia (FMD) which is a noninflammatory, nonatherosclerotic vascular disease of unknown etiology with a strong female preponderance (Baradhi & Bream, 2018). FMD can affect any artery but is typically found in the renal or cerebral vascular system. Although relatives of patients with Grange syndrome who are heterozygous carriers of pathogenic germline variants are generally asymptomatic, protein-truncating *YY1AP1* variants have been discussed as rare predisposition alleles for FMD (Guo et al., 2017).

We here present a detailed clinical description of three siblings affected with Grange syndrome highlighting that internal carotid artery (ICA) stenosis is a consistent feature usually diagnosed in the second decade of life. Furthermore, one of the biallelic *YY1AP1* splice mutations is located rather deep in the intron which emphasizes that the creation of cryptic splice sites always needs to be considered in NGS data analysis.

2 | CLINICAL REPORT

2.1 | Patient II:1

The 25-year-old index case II:1 is the first child of nonconsanguineous parents and was born at term after uneventful pregnancy (Figure 1a). She became symptomatic at the age of 15 with headaches, dysarthria, transitory disturbance of her fine motor skills, and choreatic movements. Conventional and magnetic resonance angiographies (MRA) demonstrated occlusion of the left ICA and severe stenosis of the right ICA (Figure 1e-g). Furthermore, stenoses of branches of the superior mesenteric artery and a narrowing near the origin of the lower branch of the left renal artery were identified. Symptoms of coronary artery disease were denied and echocardiography demonstrated a borderline enlargement of the left ventricle but no further heart defects. Because of chronic hypertension, three-drug combination therapy with ramipril, metoprolol, and rilmenidine was started. Extensive laboratory tests gave normal results. Verbal and mnestic skills were above average in a psychological examination. Currently, she is a well-performing university student. Neither syndactyly nor bone fragility was observed.

2.2 | Patient II:2

The 20-year-old sister (II:2) of the index patient who was also born at term after uneventful pregnancy presented with asymptomatic bilateral ICA stenosis and mild to moderate bilateral renal artery stenosis at the age of 13 and 16, respectively. Chronic hypertension was treated with a two-drug combination therapy of olmesartan and

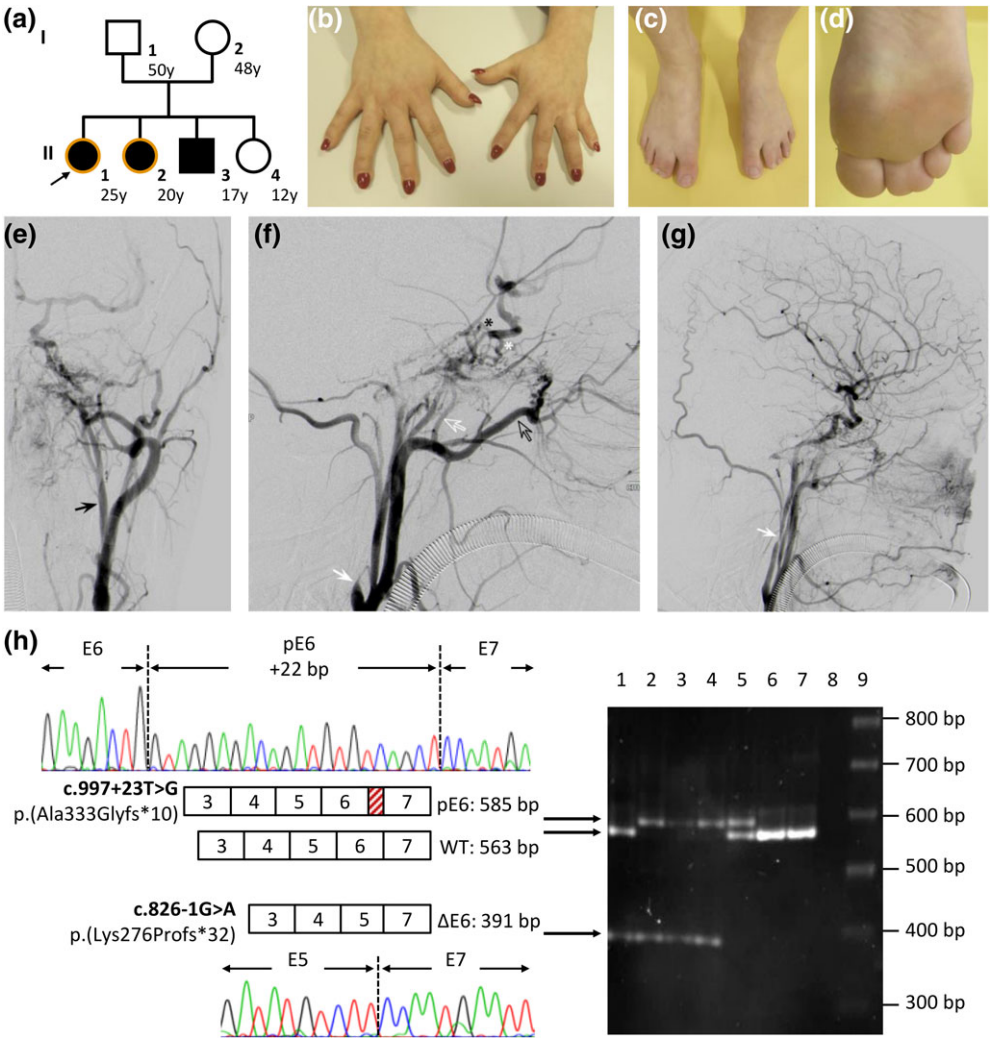

FIGURE 1 (a) Pedigree of the nonconsanguineous family with Grange syndrome. Filled symbols = individuals with verified steno-occlusive arterial lesions. Whole-exome sequenced probands are marked with orange circles. (b–d) Photographs of hands and feet of II:2. Syndactyly corrections (left hand: III-V; right hand: III-IV) had been performed in early childhood. (e–g) Digital subtraction angiography (DSA) of II:1. Left common carotid artery (CCA) in frontal (e) and lateral (f) view, right CCA in lateral view (g). Occlusion of the ICA shortly distal to the carotid bifurcation (f, white arrow). Unusually thickened ascending pharyngeal artery (e, black arrow) with a network of collaterals to the cavernous portion of the ICA via the thickened meningohypophyseal trunk (MHT, f, black asterisk) as well as via the inferolateral trunk (ILT, f, white asterisk) fed by branches of the middle meningeal artery (MMA, f, white open arrow) and the internal maxillary artery (IMA, f, black open arrow). Similar findings on the right side with severe stenosis of the ICA in the cervical portion (g, white arrow) and a very similar collateral pattern compared to the left ICA. (h) Confirmation of the predicted splice defects by RT-PCR for all affected children (2–4), their father (1) and mother (5). Lanes 6 + 7: control samples, lane 8: blank; lane 9: size marker. pE6 = c.997+23T>G allele with exonization of 22 intronic bp. Δ*E6* = c.826-1G>A allele with skipping of exon 6. E = exon

amlodipine. She also reported recurrent episodes of Raynaud's phenomenon with cold extremities and attacks of pain, tingling and numbness in fingers and toes that last minutes to hours and occur up to three times a week. While warmth and movement usually led to an improvement, prolonged episodes resulted in repeated hospitalization and intravenous alprostadil treatment. Congenital heart defects were excluded and no learning disabilities or bone fractures had been reported. Notably, II:2 presented with complete cutaneous syndactyly of the third, fourth, and fifth finger of the left hand and the third and fourth finger of the right hand which were corrected by surgery in early childhood (Figure 1b). Furthermore, she still has bilateral cutaneous syndactyly of her second and third toes (Figure 1c,d).

2.3 | Patient II:3

Patient II:3 is the 17-year-old brother of the index case and also has chronic hypertension treated with ramipril monotherapy. In line with the phenotype of his two older sisters, bilateral ICA stenosis of up to 70% was reported at the age of 15 for him. Bone fragility, learning disabilities, and symptoms of coronary heart disease or chronic mesenteric ischemia were denied. However, no invasive angiography has yet been documented for the asymptomatic young man. Like his older sister, patient II:3 has bilateral cutaneous syndactyly of his second and third toes. However, no brachydactyly or syndactyly of his fingers have been observed.

2.4 | Parents and sister II:4

The youngest sister II:4 is asymptomatic. Moderate stenosis (50%) of her left external carotid artery (ECA) had been documented once in vascular ultrasound at the age of 9 but was not seen again in follow-up examinations. Both parents are also asymptomatic and presented no steno-occlusive lesions in sonography or MRA.

3 | MATERIALS AND METHODS

3.1 | Editorial policies and ethical considerations

The study protocol was approved by the local ethics committee (University Medicine Greifswald; BB 047/14) and all patients gave their written informed consent for examination and genetic analyses.

3.2 | Genetic analyses

DNA was isolated from peripheral blood lymphocytes with the NucleoSpin Blood L kit (Machery-Nagel, Düren, Germany). The SureSelect Human All Exon v6 kit (Agilent Technologies, Santa Clara, CA) was used for exome capture and DNA libraries were sequenced on a HiSeq4000 instrument (2 × 100 cycles; Illumina, San Diego, CA). Whole-exome sequencing (WES) data were analyzed as previously described (Rath et al., 2017). *YY1AP1* variants (ENST00000368339.9) were validated by Sanger sequencing and submitted to ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). Splice predictions were calculated with Alamut Visual software v.2.10.0 (Interactive Biosoftware, Rouen, France). RNA was isolated with the Quick-DNA/RNA™ Blood Tube Kit (Zymo Research, Freiburg, Germany) and 250 ng total RNA were reverse transcribed using the SuperScript IV First-Strand Synthesis Kit (Thermo Fisher Scientific, Waltham, MA).

4 | RESULTS

Chromosome analysis of peripheral blood lymphocytes demonstrated that II:1 had a normal 46,XX karyotype. As an autosomal recessive condition was assumed to be the most plausible explanation for the complex phenotype, we performed WES for II:1 and II:2. However, no candidate gene was found when we filtered for homozygous or likely compound heterozygous variants in coding regions and conserved splice sites. Only upon inclusion of near-splice regions (± 50 bp), *YY1AP1*, *DNAH9*, and *SAMD11* were identified as candidate genes. While no phenotypes are listed in the OMIM database for the latter, *YY1AP1* is associated with Grange syndrome. These results prompted us to analyze the two identified *YY1AP1* variants in more detail. Sanger sequencing confirmed compound-heterozygosity in all affected siblings (II:1; II:2 and II:3). Both parents were shown to be heterozygous carriers. The paternal substitution c.826-1G>A is listed in gnomAD (http://gnomad.broadinstitute.org/) with only three heterozygous carriers and was predicted to induce skipping of exon 6 [r.826_997del; p.(Lys276Profs*32)]. The maternal variant c.997+23T>G, which was not listed in gnomAD, was also found in heterozygous state in a DNA sample of the youngest sister (II:4). In silico splice analyses with the Human Splicing Finder (HSF), NNSplice, Splice Site Finder-like (SSF), and MaxEnt algorithms indicated the creation of a novel donor splice site in intron 6 which had equal or even higher splice prediction scores than the wild-type splice site. A loss of YY1AP1 protein function was also assumed for this rather deep intronic variant since the use of the novel splice site is predicted to result in a frameshift due to exonization of 22 intronic nucleotides [r.997_998insGUAGGAGACAGAUGUGUCAGCU; p.(Ala333Glyfs*10)]. RT-PCR and cDNA sequencing confirmed both predicted splice defects (Figure 1h). Consequently, the *YY1AP1* variants were classified as bona fide loss-of-function mutations and as pathogenic variants for Grange syndrome according to the ACMG guidelines (Richards et al., 2015).

5 | DISCUSSION

Grange syndrome is a rare disease and our report underscores that bilateral ICA stenosis, hypertension and renal arteriopathy belong to its characteristic phenotype whereas bone fragility, intellectual disability, and congenital heart defects do not. Together with our current report, only eleven patients from six families have so far been described (Grange et al., 1998; Guo et al., 2017; Volonghi et al., 2012; Wallerstein et al., 2006; Weymann et al., 2001). Biallelic *YY1AP1* loss-of-function mutations have recently been confirmed in six of them (Guo et al., 2017). The family presented here is the first with pathogenic *YY1AP1* variants in noncoding regions and only the second with multiple affected siblings, thus illustrating intra- and interfamilial variability in Grange syndrome (Table 1).

TABLE 1 Symptoms of previously published patients with Grange syndrome and the three affected siblings of the family presented here

	Family 1				Family 2	Family 3	Family 4	Family 5	Family 6		
Patient	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**
First reported	Grange et al. (1998) DVD047[a]	Grange et al. (1998) DVD047[a]	Grange et al. (1998) DVD047[a]	Grange et al. (1998) DVD047[a]	Weymann et al. (2001) DVD093[a]	Wallerstein et al. (2006) DVD097[a]	Volonghi et al. (2012)	Guo et al. (2017) DVD112[a]	Current report II:1	Current report II:2	Current report II:3
Age at publication	29 years	27 years	18 years	15 years	15 years	3 years	18 years	?	25 years	20 years	17 years
Biallelic *YY1AP1* mutations	+[b]	+[b]	?	+[b]	+[b]	+[b]	?	+[b]	+	+	+
Arteriopathy											
ICA stenosis (age at diagnosis)	+ (26 years)	+ (26 years)	?	+ (10 years)	+ (15 years)	+ (15 years)[a]	+ (18 years)	–	+ (15 years)	+ (13 years)	+ (15 years)
Renal	(+)	+	+	+	+	+	+	–	+	+	?
Abdominal	+	+	?	+	+	–	+	–	+	–	?
Coronary	+ (32 years)[a]	–	+	+	–	?	–	?	?	?	?
Hypertension	+	+	+	+	(+)	+	+	?	+	+	+
Brachy-syndactyly	+	+	(+)[c]	(+)[c]	+	+	+	(+)[c]	–	+	(+)[d]
Congenital heart defects	+	+	+	–	(–)[e]	–	–	–	(–)[e]	–	?
Aortic dilation/ Aortopathy	–	+	–	–	–	–	+	–	–	–	?
Bone fragility	+	+	+	+	–	+	–	–	–	–	–
Developmental problems/learning disabilities	–	+	+	+	+	+	+	+	–	–	–

[a] Family numbers and additional clinical information according to Guo.
[b] Pathogenic *YY1AP1* variants according to Guo et al. (2017).
[c] Only brachydactyly.
[d] Only cutaneous syndactyly of his second and third toes.
[e] Hypertrophy of the left ventricle.
? = unknown.

All affected siblings of the current report (II:1-3) have ICA stenosis, and this has been described in five other individuals with homozygous or compound heterozygous *YY1AP1* variants (Grange et al., 1998; Guo et al., 2017; Volonghi et al., 2012; Wallerstein et al., 2006; Weymann et al., 2001). Renal artery stenosis is another frequent but also more variable feature. Some patients present with unilateral and others with bilateral renal stenosis, as early as the age of 15 months (Wallerstein et al., 2006), but also later at age 26 (Grange et al., 1998). Two of the three affected siblings in this report have renal artery stenosis, only one has arteriopathy of mesenteric vessels. Learning disabilities, brachysyndactyly, bone fragility, and congenital heart defects seem to be the most variable features of Grange syndrome. Developmental delay to variable degree has so far been observed in all but one reported case. Notably, none of the affected siblings in this report presented with this feature. Variable penetrance has also been described for syndactylies. In line with the observations of Grange et al. (1998), only two of the three affected siblings in the family presented here were born with syndactyly. Bone fragility has been reported in only two families so far (Grange et al., 1998; Wallerstein et al., 2006) and congenital heart defects have only been observed in one family (Grange et al., 1998).

Due to variable expressivity of Grange syndrome, genetic analyses of the *YY1AP1* gene may be required to confirm the diagnosis. These should cover not only all protein-coding *YY1AP1* transcript variants but also near-splice regions. Notably, the variant c.997+23T>G would not have been identified in our family and the correct diagnosis would have been missed if only exons and conserved splice sites (± 5 bp) had been analyzed. Analysis for copy number variations (CNVs) should also be part of the diagnostic workup since 23 CNV counts have already been listed in the ExAC browser for *YY1AP1* (Lek et al., 2016).

Heterozygous *YY1AP1* loss-of-function variants have also been discussed as predisposition alleles for FMD which has a prevalence of 3–4% and is therefore not a rare disease (Guo et al., 2017; Shivapour, Erwin, & Kim, 2016). Guo et al. (2017) found no increased *YY1AP1* variant burden in a cohort of 282 FMD cases but identified one heterozygous frameshift variant. Additionally, the mother of the four sibs first described by Grange et al. (1998) has unilateral renal artery stenosis and is heterozygous for the YY1AP1 variant p.(Gln242*; Guo et al., 2017). A 50% ECA stenosis has also been reported once for the youngest child of our family who is heterozygous for the maternal *YY1AP1* splice variant c.997+23T>G. In contrast, no steno-occlusive lesions had been identified in the 48-year-old mother and the 50-year-old father. Since follow-up examinations did not show any vascular lesions in II:4, it remains unclear whether the heterozygous *YY1AP1* variant is a rare FMD predisposition allele in the 12-year-old girl. Nevertheless, at-risk relatives of patients with Grange syndrome may be advised to have regular, noninvasive medical examinations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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