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, transient 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 mnemonic 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...
amlodipine. She also reported recurrent episodes of Raynaud’s phe-
nomenon 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 hospitali-
zation 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 dis-
abilities, and symptoms of coronary heart disease or chronic mesen-
teric ischemia were denied. However, no invasive angiography has yet
been documented for the asymptomatic young man. Like his older sis-
ter, 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 Sure-
Select 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.broad institute.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 het-
roygous 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_998insUGAGGAGACAGAUGUGUCAGCU; 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 disabil-
ity, 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 patho-
genic YY1AP1 variants in noncoding regions and only the second with
multiple affected siblings, thus illustrating intra- and interfamilial vari-
ability in Grange syndrome (Table 1).
| Patient | Family 1 | Family 2 | Family 3 | Family 4 | Family 5 | Family 6 |
|---------|---------|---------|---------|---------|---------|---------|
|         | I       | II      | III     | IV      | V       | VI      |
| First reported | Grange et al. (1998) DVD047<sup>a</sup> | Grange et al. (1998) DVD047<sup>a</sup> | Grange et al. (1998) DVD047<sup>a</sup> | Grange et al. (1998) DVD047<sup>a</sup> | Weymann et al. (2001) DVD093<sup>a</sup> | Wallerstein et al. (2006) DVD097<sup>a</sup> |
| Age at publication | 29 years | 27 years | 18 years | 15 years | 3 years | 18 years |
| Biallelic YY1AP1 mutations | +<sup>b</sup> | +<sup>b</sup> | ? | +<sup>b</sup> | +<sup>b</sup> | ? |
| Arteriopathy | | | | | | |
| ICA stenosis (age at diagnosis) | + (26 years) | + (26 years) | ? | + (10 years) | + (15 years) | + (15 years)<sup>a</sup> |
| Renal | + | + | + | + | + | + |
| Abdominal | + | + | ? | + | - | + |
| Coronary | + (32 years)<sup>a</sup> | - | + | - | ? | ? |
| Hypertension | + | + | + | + | (+) | + |
| Brachy-syndactyly | + | + | (+)<sup>c</sup> | (+)<sup>c</sup> | + | + |
| Congenital heart defects | + | + | + | - | (-)<sup>d</sup> | - | - |
| Aortic dilation/ Aortopathy | - | + | - | - | - | + |
| Bone fragility | + | + | + | - | - | - |
| Developmental problems/learning disabilities | - | + | + | + | + | - |

<sup>a</sup> Family numbers and additional clinical information according to Guo.
<sup>b</sup> Pathogenic YY1AP1 variants according to Guo et al. (2017).
<sup>c</sup> Only brachydactyly.
<sup>d</sup>Only cutaneous syndactyly of his second and third toes.
<sup>e</sup>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 syndactyly. 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.

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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