ARTICLE

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TAB2 deletions and variants cause a highly recognisable syndrome with mitral valve disease, cardiomyopathy, short stature and hypermobility

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Deletions that include the gene *TAB2* and *TAB2* loss-of-function variants have previously been associated with congenital heart defects and cardiomyopathy. However, other features, including short stature, facial dysmorphisms, connective tissue abnormalities and a variable degree of developmental delay, have only been mentioned occasionally in literature and thus far not linked to *TAB2*. In a large-scale, social media-based chromosome 6 study, we observed a shared phenotype in patients with a 6q25.1 deletion that includes *TAB2*. To confirm if this phenotype is caused by haploinsufficiency of *TAB2* and to delineate a *TAB2*-related phenotype, we subsequently sequenced *TAB2* in patients with matching phenotypes and recruited patients with pathogenic *TAB2* variants detected by exome sequencing. This identified 11 patients with a deletion containing *TAB2* (size 1.68–14.31 Mb) and 14 patients from six families with novel truncating *TAB2* variants. Twenty (80%) patients had cardiac disease, often mitral valve defects and/or cardiomyopathy, 18 (72%) had short stature and 18 (72%) had hypermobility. Twenty patients (80%) had facial features suggestive for Noonan syndrome. No substantial phenotypic differences were noted between patients with deletions and those with intragenic variants. We then compared our patients to 45 patients from the literature. All literature patients had cardiac diseases, but syndromic features were reported infrequently. Our study shows that the phenotype in 6q25.1 deletions is caused by haploinsufficiency of *TAB2* and that *TAB2* is associated not just with cardiac disease, but also with a distinct phenotype, with features overlapping with Noonan syndrome. We propose the name "*TAB2*-related syndrome".

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INTRODUCTION

The gene *TAB2* (TGFβ-activated kinase 1 binding protein 2, MIM*605101) is mapped to chromosome 6q25.1. Haploinsufficiency of *TAB2* is associated with congenital heart defects (CHD) [1] and cardiomyopathy [2]. Other features have only been described occasionally, including short stature, facial dysmorphisms, connective tissue abnormalities and a variable degree of developmental delay [2, 3]. Cheng et al. reported 6q25.1 deletion patients who showed features overlapping with Noonan syndrome (NS) [2].

NS is a relatively common genetic disorder characterised by typical facial dysmorphisms, developmental delay, short stature and cardiac abnormalities (e.g., pulmonary valve stenosis, atrial septal defects (ASDs) and cardiomyopathy). More than 15 genes have been described in association with NS, all part of the Ras/MAPK (mitogen-activated protein kinase) signal transduction pathway. However, in approximately 20% of clinically diagnosed NS patients, no (likely) pathogenic variant in these genes is detected [4].

In a large social media-based project on chromosome 6 aberrations, we observed that individuals with a 6q25.1 deletion shared a distinct phenotype that could most likely be attributed to *TAB2* haploinsufficiency. To explore this, we recruited a second cohort with matching phenotypes in whom we sequenced *TAB2* and further included patients in whom a pathogenic *TAB2* variant had been found by exome sequencing. This enabled us to further delineate the *TAB2*-related phenotype.

METHODS

We describe patients with deletions containing *TAB2* and patients with intragenic variants in *TAB2*. We compare these patients with literature case reports and define the *TAB2*-related phenotype.

Cohort 1: Patients with chromosome 6 aberrations recruited via social media

Patients were recruited via the Chromosome 6 Project, a parent-driven social media-based research project into chromosome 6 aberrations, as

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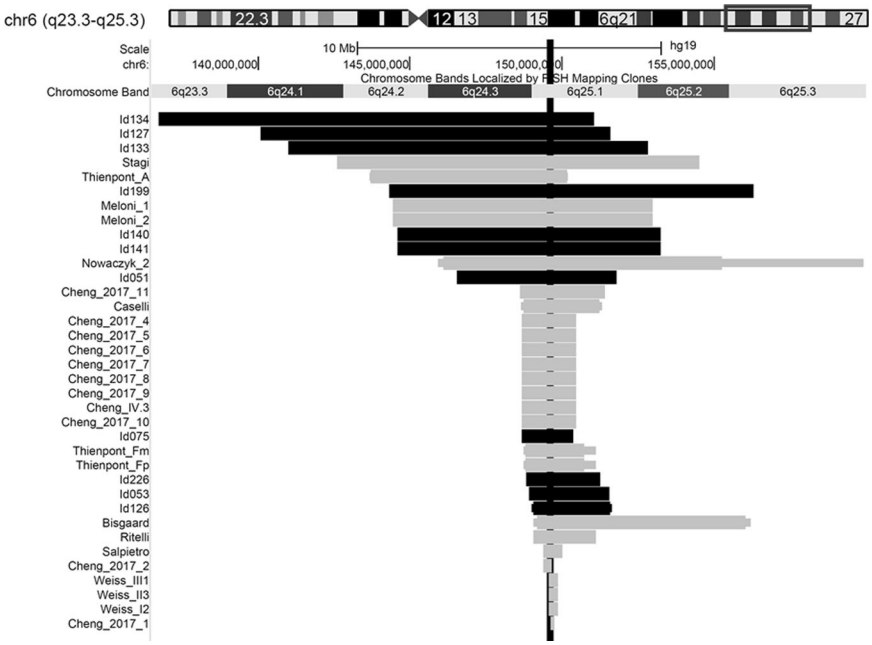

Fig. 1 Overview of all deletions leading to our candidate gene *TAB2*. Deletions including the gene *TAB2* are shown for our social media cohort (horizontal black bar) and the literature (horizontal grey bar). The minimum and maximum extent of the deletions are shown, if these were known. The smallest region of deletion overlap only includes our candidate gene *TAB2* (vertical black bar). Deletions visualised using the UCSC genome browser (https://genome.ucsc.edu). Literature cases were derived from 11 reports [1–3, 11–18]. See Tables S1 and S2 for details.

described previously [5]. Patients or their legal representatives were approached to participate via a Chromosome 6 Facebook group, Twitter (@C6study) and our website (https://www.chromosome6.org). Inclusion criteria were an isolated chromosome 6 aberration and availability of a microarray report. Microarray analyses were performed in diagnostic laboratories using different platforms, as specified in Table S1. Microarray results were converted to GRCh37/hg19 using the UCSC LiftOver Tool and visualised using the UCSC Genome Browser (http://genome.ucsc.edu).

Information on the phenotype was collected directly from patients or their legal representatives via a multilingual Chromosome 6 Questionnaire constructed using the MOLGENIS toolkit [6]. The questionnaire contains questions on congenital abnormalities, relevant dysmorphic features, development, behaviour and health-related problems (including cardiac disease) of the individual. Consent for publication was obtained from all participants. Consent for the use of photographs was optional. After consent, additional information was requested from the treating physicians. By focussing on patients with heart defects, we found a shared deletion region in 6q25.1.

Literature review chromosome 6q deletions

We used PubMed and Google Scholar to search for information on previously reported patients with a chromosome 6q deletion. Search terms included: chromosome 6q, 6q deletion, monosomy 6q and 6q*. Only publications reporting the current diagnostic standard procedures (including microarray results) were included.

By combining genotype data of patients with a heart defect from the Chromosome 6 Project with genotype data from patients described in the literature, we found a smallest region of deletion overlap within chromosome band 6q25.1. The only gene within this region is *TAB2*. For the present study, we only included patients and literature cases with a deletion that includes *TAB2* (further referred to as: *TAB2* deletion). Figure 1 visualises the microarray analysis results and the smallest region of overlap including *TAB2*. We then delineated a *TAB2* deletion phenotype for this group of individuals.

Data collected from *TAB2* deletion patients in Cohort 1 was submitted to the DECIPHER database (decipher.sanger.ac.uk), IDs 425375 and 425379-425388.

Cohort 2: Patients with *TAB2* variants

After recognising the *TAB2* deletion phenotype, we identified families with normal microarray results but a similar phenotype in our own institution. After confirming that they had *TAB2* variants, we recruited other patients with *TAB2* variants identified by exome sequencing at two other Dutch hospitals. Consent was obtained for publication and for publication of photographs, if applicable.

TAB2 variants were submitted to the Global Variome shared LOVD and can be accessed here: databases.lovd.nl/shared/references/DOI:10.1038/s41431-021-00948-0.

Exome sequencing was performed in the genomic diagnostic laboratories of the three Dutch university hospitals, as described previously [7–9]. Sanger sequencing of *TAB2* was performed in the Radboud University Medical Center, Nijmegen, the Netherlands. DNA was isolated from leucocytes by standard methods. The coding region and exon–intron boundaries of *TAB2* (GenBank: NM_015093.5) were amplified from genomic DNA. Sanger sequencing was performed using the BigDye Terminator Sequencing Kit and ABI 3730 XL (Applied Biosystems). Primers are available on request.

Literature review *TAB2* variants

PubMed and Google Scholar were used to search for previously reported patients with a pathogenic *TAB2* variant. Search terms included: *TAB2* and variant/mutation.

Clinical scoring system NS

The clinical scoring system for NS published by Van der Burgt et al. [10] was used to compare the clinical features of our patients with NS. The system scores six criteria, one of which is a typical or suggestive face for NS. This facial criterion includes six characteristics: broad forehead, hypertelorism, downslanting palpebral fissures, ptosis, low set and posteriorly angulated ears and broad and/or short neck. If all six facial characteristics were present in our patients, the face was defined as “typical”. If four of the six facial characteristics were present, the face was defined as “suggestive” for NS. In all patients, the craniofacial phenotype was evaluated by a clinical geneticist either in the outpatient clinic or using photographs.

RESULTS

We describe 25 cases from 16 families with *TAB2* deletions ($n = 11$) or *TAB2* variants ($n = 14$) and compare these with data from 45 literature cases.

Table 1. Genotype and clinical characteristics in *TAB2* deletions and variants.

	***TAB2* deletion cohort** ***n* = 11**	***TAB2* deletion literature** ***n* = 25**	***TAB2* variant cohort** ***n* = 14**	***TAB2* variant literature** ***n* = 22**
Deletion size	8.67 Mb (1.68–14.31)[a]	1.76 Mb (0.12–11.92)[a]		
Age last follow up	4 years (8 mos to 40 yrs)[a]	9.5 years (15 days to 66 yrs)[a]	11.5 years (2–46)[a]	30 years (2–75)[a]
Gender (F/M)	9/2	16/9	8/6	12/10
Birth weight	10th centile (3–90)[a]	25th centile (2–75)[a]	10th centile (150)[a]	
Height	−2.5 SD (−3.8 to −1)[a]	3rd centile (1–27)[a]	−2.5 SD (−4.8 to −0.3)[a]	
Head circumference	+0.9 SD (−3 to +1.5)[a]	50th centile (1–97)[a]	−0.8 SD (−1.5 to 0)[a]	
Facial characteristics				
Broad forehead	11/11	15/25	13/14	9/22
Hypertelorism	10/11	6/25	4/14	2/22
Up/downslant	6/11	7/25	7/14	4/22
Ptosis	10/11	13/25	11/14	9/22
Ears, abnormal position	10/11	10/25	8/14	2/22
Broad short neck	1/11	2/25	3/14	1/22
Heart				
CHD/TAA	7/11	24/25	12/14	19/22
Cardiomyopathy	4/11	7/25	4/14	8/22
Arrhythmia	2/11	4/25	2/14	4/22
Connective tissue/skeletal				
Hypermobility	7/11	8/25	11/14	5/22
Pedes planovalgi	3/11	2/25	10/14	2/22
Pectus excavatum	1/11	1/25	6/14	0/22
Hypotonia	9/11	8/25	2/14	2/22
Hearing loss	5/11	1/25	5/14	5/22
Developmental delay	7/11	12/25	4/14	0/22
Definite NS[b]	6/11		7/14	

For more detailed overview see Table S1: *TAB2* deletion cohort, Table S2: *TAB2* deletion literature, Table S3: *TAB2* variant cohort and Table S4: *TAB2* variant literature.

CHD congenital heart disease, *F* female, *M* male, *NS* Noonan syndrome, *SD* standard deviation, *TAA* thoracic aorta aneurysm.

[a]Median (range)

[b]See Table S5 for details.

Identifying the candidate gene *TAB2* in 6q25.1 deletion patients

Data from 36 deletion patients (Chromosome 6 Project, $n = 11$; literature case reports, $n = 25$) showed a cluster with a shared phenotype of CHD and short stature (<−2 SD) in the region 6q25.1. The size of the deletions ranged from 0.12 to 14.31 Mb. The smallest region of deletion overlap led to our candidate gene *TAB2* (see Fig. 1).

Patient characteristics of cohort 1: *TAB2* deletion cohort

The data on 11 patients (9 female, 2 male, median age 4 years (range 8 months to 40 years)) from 10 families collected via the Chromosome 6 Project are summarised in Table 1 (details in Table S1). Eight of these 11 patients (73%) had cardiac anomalies. Six had congenital valve defects involving one or more valves, with the mitral valve involved in five patients. An additional ASD and/or ventricular septal defect (VSD) was present in four patients. At age 1 week, patient Id134 had surgical closure of her VSD and a coarctation of the aorta was repaired. Four patients had cardiomyopathy, three in combination with a structural heart defect. Two children, Id134 and Id133, died due to dilated cardiomyopathy (DCM) at age 8 months and 4 years, respectively. All patients had similar facial characteristics including a broad forehead (100%), hypertelorism (91%), ptosis (91%) and low set ears (91%) (Fig. 2). Eight patients (73%) had a short stature, and in two this was disproportionate (short limbs). One patient was eligible for growth hormone therapy. Connective tissue abnormalities, including joint hypermobility, umbilical and inguinal hernias and skeletal and/or skin abnormalities, were reported in nine patients (82%) (data in Table S1). Nine patients had hypotonia (82%), five reported hearing loss (45%) and seven had mild-to-severe developmental delay (64%).

Literature on *TAB2* deletions

Twenty-five patients with a *TAB2* deletion were described in 11 literature case reports [1–3, 11–18]. The literature data is summarised in Table 1 (details in Table S2). All patients had a cardiac anomaly (100%), and 24 had a structural malformation. Single or multiple valve defects were seen in 20 patients, and most had a mitral valve defect, often in combination with another valve defect. Thirteen patients had an (additional) ASD, VSD, or both. Hypoplastic left heart syndrome (HLHS) was reported in one patient (IV.3), who died shortly after birth [13]. Cardiac surgery was performed in five patients. Three young children had CHD repair including the closure of a VSD [2, 16, 17]. The pulmonic valve was also replaced for one of them. Her grandmother had a mitral valve replacement at age 22 years [17]. The fifth patient underwent aortic root repair and aortic valve replacement at age 51 years [2]. Seven patients developed a cardiomyopathy. Dysmorphic facial characteristics were reported in 22 patients (88%). Twenty-one patients had a short stature (84%). A patient reported by Stagi et al. received growth hormone therapy, with good results

Fig. 2 Clinical photographs of patients with a *TAB2* deletion. Top row (left to right): patient Id134 at age 6 months, patient Id127 at age 4 years and patient Id133 at age 4 years. Middle row (left to right) patient Id199 at age 3 years, patient Id141 at age 6 years and patient Id140 at age 34 years. Bottom row (left to right): patient Id226 at age 2 years, patient Id053 at age 6 years and patient Id126 at age 8 years. Note the characteristic facial phenotype: a broad forehead, hypertelorism, ptosis, low set ears and an upslant in most patients. Written consent was given to the authors to publish the patient's photographs.

reported [18]. Connective tissue abnormalities were reported in 12 patients (48%) (Table S2), 8 patients (32%) had hypotonia and 12 patients (48%) had mild-to-moderate developmental delay.

Patient characteristics of cohort 2: *TAB2* variants cohort

The data collected for 14 individuals (8 female, 6 male, median age 11.5 years (range 2–46 years)) from six families with pathogenic *TAB2* variants are summarised in Table 1 (details in Table S3).

After reviewing the facial phenotypes of the cluster of patients with CHD from the Chromosome 6 Project, author WK recognised the 6q25.1 deletion phenotype in two families (C and D) known at the department in Groningen. The patients in these families had normal microarray results.

The proband (D6) of family D was the first patient clinically suspected of a pathogenic *TAB2* variant. Family D is a three-generation family with six individuals affected with mitral and pulmonary valve defects, disproportionate short stature, hypermobility, pes planus and hearing loss. NS had been considered, but no variants in known NS-related genes were detected. Exome sequencing with the request to first analyse *TAB2* revealed a novel nonsense variant (c.899 C > A (p.(Ser300*))) in the proband, and Sanger sequencing confirmed the variant in another four affected family members. The deceased affected grandmother was an obligate carrier, and the variant was excluded in the grandfather and one non-affected child (Fig. S1D).

In family C, *TAB2* was directly analysed by Sanger sequencing after clinical recognition. This is a two-generation family with three affected individuals with mitral valve defects, aortic dilatation, disproportionate short stature, hypermobility or hypotonia, pes planus and pectus excavatum. Sanger sequencing revealed a novel frameshift variant, predicted to result in a premature stop in *TAB2* (c.885_886del (p.(Pro296fs))), in all three affected family members (Fig. S1C).

Consecutively, another four novel truncating *TAB2* variants were detected by exome sequencing in families A, B, E and F (Table S3). Their pedigrees are shown in Fig. S1, and the positions of the variants are shown in Fig. S2.

In summary, in our six families with a *TAB2* variant, 12 out of 14 patients had heart disease (86%). In one patient, a cardiac workup was not performed. Nine patients had mitral valve defects, and four also had a cardiomyopathy. Thoracic aortic aneurysms were reported in three patients from two families. Patient B1 had a VSD, which was surgically closed at age 7 months. Patient D1 had an aortic valve replacement at age 39 years and died at age 46 years, cause unknown. All 14 patients share specific facial characteristics: broad forehead (93%), up/downslant (50%), ptosis (79%) and low set ears (57%) (Fig. 3). Ten patients (71%) had short stature, mostly disproportionate (relatively short limbs). Connective tissue abnormalities were

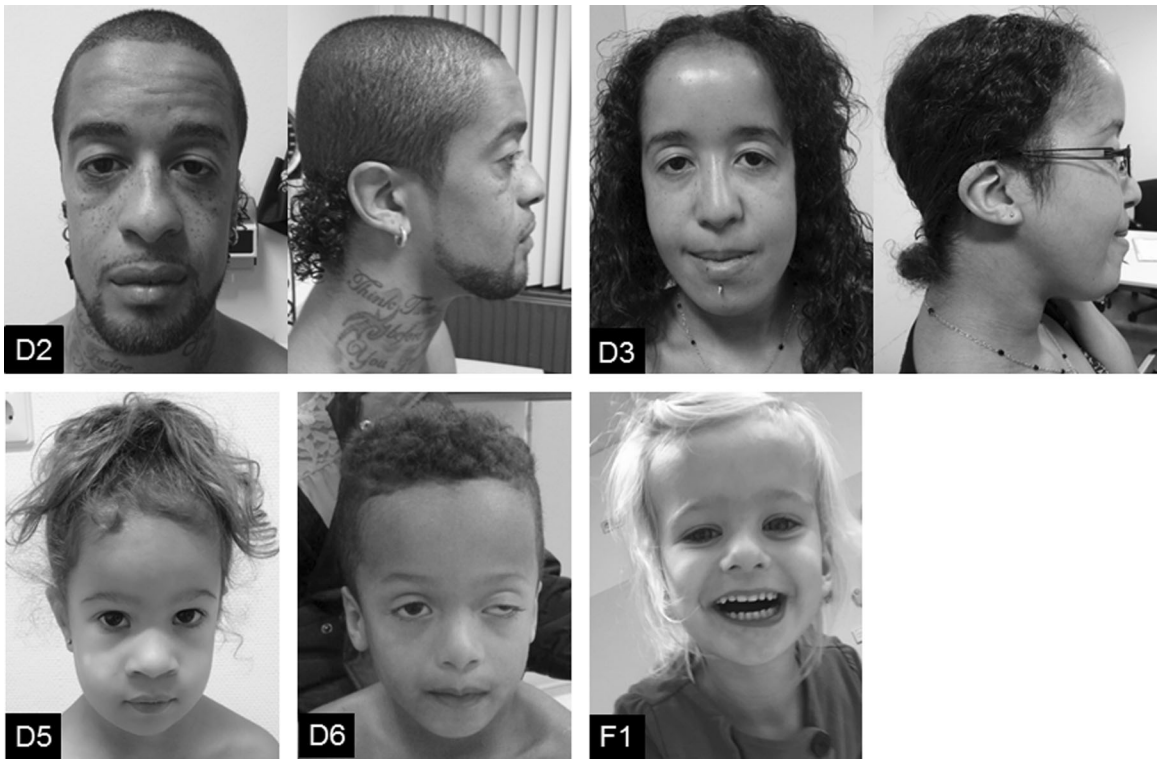

Fig. 3 Clinical photographs of patients with a *TAB2* variant. Top row (left to right): Patient D2 at age 32 years and patient D3 at age 24 years. Bottom row (left to right): patient D5 at age 4 years, patient D6 at age 5 years and patient F1 at age 3 years. Note the characteristic facial phenotype: a broad forehead, an up/downslant, ptosis and low set ears. Written consent was given to the authors to publish the patient's photographs.

seen in 13 patients (93%) (Table S3): 11 had joint hypermobility, 10 had pes planus and six had pectus excavatum. Hearing loss was reported in five patients (36%) and mild developmental delay in four (29%).

Literature on *TAB2* variants

Four familial and four sporadic *TAB2* variants in 20 patients were reported in the literature (Fig. S2) [1, 3, 19–23]. The data extracted from the literature reports are summarised in Table 1 (details Table S4). Six variants were truncating, two were missense variants. All 20 literature patients had cardiac diseases (100%). Fourteen patients had mitral valve defects, eight had cardiomyopathy and five had both. One patient had a mitral valve replacement at age 75 years, and his son had a mitral and aortic valve replacement at age 51 years [19]. A 39-year-old man had DCM and a pulmonary artery aneurysm. This aneurysm was resected at age 35 years and a pulmonary homograft valve was implanted. Due to heart failure, he is now on the heart transplant list [21]. One girl was diagnosed with DCM at age 7 months. She received a heart transplant at age 9 months and died at age 2.5 years [22]. A 60-year-old man and a 61-year-old woman died due to sudden cardiac arrythmia and heart failure, respectively [1, 3]. Ten of the 20 patients (50%) were reported to have dysmorphic facial characteristics, 10 had a short stature (50%) and seven had connective tissue abnormalities (35%) (Table S4). Hearing loss was reported in four patients (20%). Developmental delay has not been reported.

In addition to the 20 patients discussed above, Wade et al. described two unrelated individuals with missense variants with a completely different phenotype of frontometaphyseal dysplasia type 3 that was suggested to be caused by gain-of-function variants in *TAB2*. These patients did not display cardiac anomalies or features overlapping with NS [24, 25].

Clinical overlap with NS in patients with a *TAB2* variant or deletion

In four of our families with a *TAB2* variant, NS had been considered as a diagnosis, but no variants in NS-associated genes were detected. In one of our *TAB2* deletion patients, NS was considered as a diagnosis, but testing was not performed after the abnormal microarray result. In the literature, NS-associated genes were tested in two *TAB2* variant cases and two *TAB2* deletion cases, but no pathogenic variants were identified [3, 13, 23] (Tables S1–S4).

We retrospectively scored all patients with a *TAB2* variant or deletion in our cohorts for the diagnostic criteria of NS. Twenty patients (80%) had facial features suggestive for NS. Six *TAB2* deletion patients (55%) and seven *TAB2* variant patients (50%) fulfilled the criteria for definite NS (Table S5).

Combining the data from our two cohorts and the literature reports ($n = 70$), we observed cardiac disease in 93% of patients, mainly mitral valve anomalies (66%) and/or cardiomyopathy (34%); short stature in 70%; connective tissue features including hypermobility in 59% and dysmorphic facial features in 81%. Surgical interventions for cardiac anomalies are reported in 13 patients (19%). Four patients died from the (late) effects of structural heart disease, and three patients with DCM died in infancy.

DISCUSSION

According to Online Mendelian Inheritance in Man (OMIM), *TAB2* is associated with non-syndromic CHD (MIM#614980) [26]. However, our study clearly demonstrates that the phenotype is not restricted to the heart but is a recognisable syndrome that shows considerable overlap with NS. Our study further demonstrates how social media recruitment can be a very powerful tool to delineate rare diseases.

Only two earlier reports discussed the possibility of a broader phenotype [2, 3]. Other reports occasionally mentioned dysmorphic features, but a *TAB2*-related syndrome was not considered. This may be due to a lack of detailed clinical data in these other reports, or to variability in gene expression. A milder phenotype, restricted to the heart, in the two missense variants reported by Thienpont et al. cannot be excluded [1]. As the features we describe are recurrently seen in both *TAB2* deletion patients and patients with pathogenic *TAB2* variants, they may be caused by loss-of-function (LOF) of one *TAB2* allele. However, the genetic heterogeneity in *TAB2*-related disease is intriguing. The completely different phenotypes reported by Wade et al. associated with *TAB2* missense variants are assumed to be caused by gain of function of *TAB2* [24, 25].

Data from 70 patients (25 described by us and 45 from literature) demonstrates that the recurrent *TAB2*-related phenotype should be included in the differential diagnosis of NS.

Heart disease is a key feature of the *TAB2*-related phenotype. All patients with *TAB2* variants or deletions described in the literature had cardiac anomalies. Our data confirm that cardiac disease is a frequent feature, however, five of our patients did not show cardiac disease (three patients with a *TAB2* deletion and two with a *TAB2* variant). An investigative and/or publication bias towards patients with heart disease in literature and the limited information on genotypes and phenotypes of relatives in most reports may explain the difference. However, this difference may also partly be due to the slightly younger median age of our patients compared to the literature (4 vs 9.5 years in deletions; 11.5 vs 30 years in variants), which may have resulted in a lower number of patients with (late onset) cardiomyopathy. In our cohorts and in the literature, a variety of cardiac anomalies are observed, with mitral valve defects reported most frequently, often in combination with defects of one or more of the other valves. ASDs and/or VSDs are reported more often in *TAB2* deletions, both in our cohort and in the literature, in comparison to patients with a *TAB2* variant, suggesting involvement of other genes. Interestingly, *CITED2* (CBP/p300-interacting transactivator, with glu/asp-rich c-terminal domain 2, MIM*602937, 6q24.1) is known to be associated with ASDs and VSDs, but this gene was only deleted in one patient (Id134). In the other 16 *TAB2* deletion patients with ASDs and/or VSDs, the deletion did not include genes known to cause cardiac defects other than *TAB2*. Furthermore, cardiomyopathy, often DCM, is present in 34% of the patients overall, and the prevalence could actually be higher, as cardiomyopathy can occur later in life and most of the reported patients are children.

The most severe structural cardiac disease was reported by Cheng et al. [2, 13]. Two children in one family were born with HLHS and died shortly after birth. One of these children had the familial *TAB2* deletion; the other was not tested. Considering that this is the only report of HLHS associated with a *TAB2* variant, HLHS may either be the most severe presentation in the spectrum of cardiac valve defects, or another genetic variant might be involved in this family. Unfortunately, exome sequencing was not performed [2, 13].

The cardiac anomalies observed in our cohorts and in previously reported patients required surgery in some cases and caused early death in a few patients. *TAB2*-associated cardiac anomalies appear more severe than those observed in NS. In addition, the mitral valve is more frequently involved in *TAB2*-associated cardiac anomalies as opposed to the pulmonary valve in NS.

Short stature (disproportionate in most reports) is the second most frequent characteristic of the *TAB2*-related phenotype. Apart from *TAB2*, two genes have been suggested as candidate genes for short stature in deletion cases: *LATS1* (large tumour suppressor kinase 1, MIM*603473, 6q25.1) [15] and *PLAGL1* (pleomorphic adenoma gene-like 1, MIM*603044, 6q24.2) [18]. Because the prevalence of (disproportionate) short stature was equally high in our two cohorts, we conclude that the short stature in patients with 6q25.1 deletions is most likely caused by *TAB2* haploinsufficiency.

Typical facial dysmorphisms are also part of the *TAB2*-related phenotype and were similar in our two cohorts. Twenty of the 25 patients (80%) had a face suggestive of NS. Facial characteristics overlapping with those in NS were reported previously, most often in *TAB2* deletion patients. Connective tissue anomalies, most often joint hypermobility, were reported in 82% of our *TAB2* deletion patients and 93% of our *TAB2* variant patients. In the literature, this was 48% and 35%, respectively. The gene *UST* (uronyl 2-sulfotransferase, MIM*610752) has been suggested to cause the connective tissue anomalies seen in *TAB2* deletion patients [15]. However, we do not find clear differences between our two cohorts, which suggests that LOF of *TAB2* is the most probable explanation for these features.

Developmental delay in individuals with a deletion including *TAB2* is variable. Moderate-to-severe developmental delay was only reported in individuals with a deletion larger than 6.47 Mb. This might very well be caused by the LOF effect of other genes or regulatory sequences involved in the deletion. However, four patients in our *TAB2* variant cohort also had mild developmental delay. This may suggest *TAB2* plays a role in psychomotor development, but the numbers are too low to be conclusive given that this has not been reported in 20 patients with *TAB2* variants in previous publications.

Finally, hearing loss and decreased vision are present in several of our patients and the patients reported in the literature. It remains unclear whether these problems are part of the *TAB2*-related phenotype, or coincidental, as the numbers are low and detailed clinical data are not available [21].

In our two cohorts, all patients had a syndromic phenotype. Non-syndromic *TAB2*-associated CHD has been reported in the literature, but these studies unfortunately do not include clinical photographs [1, 17, 20]. The primary focus on heart disease in these studies might have hampered the recognition of other mild features, including facial characteristics, connective tissue abnormalities or developmental delay, although we cannot rule out substantial variability in expression.

We confirmed that the *TAB2*-related phenotype has overlap with NS, as suggested previously by Cheng et al. in patients with *TAB2* deletions [2]. More than half of our patients could be clinically diagnosed with definite NS based on the scoring system of Van der Burgt et al. [10]. The face was suggestive of NS in 80%. However, in our opinion the typical NS "Gestalt" is different from the facial features in the *TAB2*-related phenotype, especially the eyes appear different, with quite narrow palpebral fissures as most clearly shown in patients Id140, Id141 and F1. Our patients also present with hypermobility and increased skin folds, which is not typical for NS. Since the *TAB2*-related phenotype is equally present in both *TAB2* deletion patients and patients with pathogenic *TAB2* variants, we expect the *TAB2*-related phenotype to be a result of LOF of *TAB2*.

Several functional and expression studies support that a *TAB2* variant may be related to NS/RASopathies. An in-silico analysis showed that *TAB2* is in strong co-expression with *SOS1* (Fig. S3) [27], one of the many genes of the MAPK pathway known to be associated with NS [28]. *TAB2* is also an activator of *MAP3K7* (mitogen-activated protein kinase kinase kinase 7, MIM*602614, coding for the TAK1 protein), another member of the MAPK pathway. These data suggest that the *TAB2*-related phenotype may be associated with the RASopathies, although further research is needed to confirm this hypothesis. The role of Tab2 in cardiac development has also been shown in mice and zebrafish [1, 29]. In humans, *TAB2* is expressed in endothelial cells of the ventricular trabeculae and the endocardial cushions of human embryos [1]. The endocardial cushions are involved in cardiac valve formation, and cardiac valve abnormalities are the

most common cardiac problem in our *TAB2*-related phenotype. Morlino et al. demonstrated that a *TAB2* variant (c.1398dup (p.(Thr467fs))) encodes a truncated protein that is unable to bind to TAK1 [27]. They showed reduced proliferation in patient's fibroblasts, which could explain some clinical features we observed, such as short stature, skeletal underdevelopment and facial characteristics [30].

Zhang et al. have shown that TAB2 expression is essential for prolonged activation of the protein TAK1 [31]. Haploinsufficiency of *TAB2* might therefore result in a decreased activation of TAK1. An intronic variant in *MAP3K7* causing a two amino-acid insertion in the kinase domain of TAK1 has been reported in a patient with cardiospondylocarpofacial syndrome (CSCFS, MIM#157800), which has overlap with the *TAB2*-related phenotype, specifically polyvalvular cardiac anomalies, short stature and connective tissue abnormalities [30]. Thus far it is claimed that *MAP3K7* deletion patients do not have any overlap with CSCFS [30]. However, we previously reviewed patients with deletions including *MAP3K7* ($n = 14$) and found characteristics similar to those seen in the *TAB2*-related phenotype: short stature, hypermobility and cardiac anomalies (mainly ASDs) but no cardiac valve defects [5]. All the *MAP3K7* deletions we reviewed were >6.43 Mb and contained other genes that might explain the phenotype. Since no valve defects are seen in patients with a *MAP3K7* deletion, the interaction of *TAB2* and *MAP3K7* must be more complex than a simple lack of activation of *MAP3K7*.

Our report of 25 patients with *TAB2* variants or deletions has some limitations. The data of deletion patients collected via online recruitment is self-reported information provided by parents, which may not always be accurate. However, these data have been compared with information collected from medical professionals and appear to be highly consistent (unpublished data). Another limitation may be that our *TAB2* variant patients were collected retrospectively. Therefore, data on the overall prevalence of *TAB2* variants is not available. In addition, the reported phenotype might be biased towards the more severe end of the spectrum, as these patients are more likely to undergo exome sequencing or microarray testing.

We further demonstrated that recruiting patients via social media can result in efficient case finding. The data on 6q25.1 deletion patients collected via social media was sufficiently detailed and complete to allow us to recognise this phenotype in families with normal microarray results. We were then able to prove that *TAB2* is responsible for the phenotype in 6q25.1 deletion patients by identifying *TAB2* variants in six families. Combining data from our cohorts and from literature allowed us to delineate the *TAB2*-related phenotype.

Based on this phenotype, we recommend that *TAB2* be added to the gene panels used in genetic work-up of patients with congenital heart disease, cardiomyopathy, short stature and connective tissue disease, and those suspected for NS, as well as to neonatology rapid sequencing panels. We also recommend CNV analysis for deletions including *TAB2* in these patients. Based on the clinical and molecular relationship with *MAP3K7*, analysis of this gene should also be considered in these patients.

For individuals with a newly detected *TAB2* variant or deletion, we recommend cardiac evaluation followed by regular follow-up, as valvular heart disease, thoracic aneurysm and cardiomyopathy may manifest later in life. We also recommend evaluation of overall development, hearing, vision and connective tissue abnormalities. Genetic counselling should be offered to all individuals with a known *TAB2* variant or deletion. Further studies are required to delineate the role of *TAB2* in causing short stature and to examine the feasibility of growth hormone therapy.

In conclusion, we successfully used social media to delineate the *TAB2*-related phenotype as a syndrome with cardiac disease, short stature, specific facial appearance, connective tissue abnormalities (mainly hypermobility) and occasionally mild intellectual disability. This phenotype may be caused by heterogeneous genetic conditions, either a deletion including the gene *TAB2*, or a truncating, or missense variant in *TAB2*. We propose naming this phenotype "*TAB2*-related syndrome". We recommend adding *TAB2* to appropriate gene panels and testing for deletions including *TAB2* in patients who have congenital heart disease, cardiomyopathy, short stature and/or connective tissue disease and/or are suspected for NS. We also recommend thorough evaluation and follow-up in patients diagnosed with a *TAB2*-related syndrome, including regular screening for cardiomyopathy and thoracic aneurysm. More data are needed to develop detailed follow-up guidelines for the *TAB2*-related syndrome.

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AUTHOR CONTRIBUTIONS

Conceptualization: AE, CMAR, WSK; Data curation: AE; Funding acquisition: AE, BF, CMAR, WSK; Investigation: AE, CMAR, WSK; Methodology: AE, CMAR, WSK; Resources: AE, EKSML, BF, PAT, KL, BBAV, TD, YJV, TR, MPB, MTRR, WSK; Supervision: WSK; Visualization: AE, PD; Writing – original draft: AE; Writing – review & editing: AE, EKSML, BF, PAT, KL, BBAV, TD, YJV, TR, MPB, MTRR, CMAR, WSK.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

The accredited Medical Ethics Review Committee of the University Medical Center Groningen waived full ethical evaluation because, according to Dutch guidelines, no ethical approval is necessary if medical information that was already available is used anonymously and no extra tests have to be performed.

ADDITIONAL INFORMATION

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