Pathogenic variants causing \textit{ABL1} malformation syndrome cluster in a myristoyl-binding pocket and increase tyrosine kinase activity

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Abstract

\textit{ABL1} is a proto-oncogene encoding a nonreceptor tyrosine kinase, best known in the somatic \textit{BCR-ABL} fusion gene associated with chronic myeloid leukaemia. Recently, germline missense variants in \textit{ABL1} have been found to cause an autosomal dominant developmental syndrome with congenital heart disease, skeletal malformations and characteristic facies. Here, we describe a series of six new unrelated individuals with heterozygous missense variants in \textit{ABL1} (including four novel variants) identified via whole exome sequencing. All the affected individuals in this series recapitulate the phenotype of the \textit{ABL1} developmental syndrome and additionally we affirm that hearing impairment is a common feature of the condition. Four of the variants cluster in the myristoyl-binding pocket of \textit{ABL1}, a region critical for auto-inhibitory regulation of the kinase domain. Bio-informatic analysis of transcript-wide conservation and germline/somatic variation reveals that this pocket region is subject to high missense constraint and evolutionary conservation. Functional work to investigate \textit{ABL1} kinase activity in vitro by transient transfection of HEK293T cells with variant \textit{ABL1} plasmid constructs revealed increased phosphorylation of \textit{ABL1}-specific substrates compared to wild-type. The increased tyrosine kinase activity was suppressed by imatinib treatment. This case series of six new patients with germline heterozygous \textit{ABL1} missense variants further delineates the phenotypic spectrum of this condition and recognises microcephaly as a common finding. Our analysis supports an \textit{ABL1} gain-of-function mechanism due to loss of auto-inhibition, and demonstrates the potential for pharmacological inhibition using imatinib.

Introduction

\textit{ABL1} is a proto-oncogene encoding a nonreceptor tyrosine kinase with diverse roles in cytoskeleton remodelling and the DNA damage response [1]. It is best known as part of the somatic \textit{BCR-ABL} fusion gene in the Philadelphia

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chromosome, associated with chronic myeloid leukaemia (CML) and acute lymphocytic leukaemia (ALL) [2].

ABL1 spans 170 kb of chromosome 9q34.12, comprising 11 exons. It has two isoforms owing to use of alternative first exons. The longer transcript (NM_007313), encodes 19 additional N-terminal residues involved in auto-inhibition of the ABL1 kinase.

Recently, Wang et al. [3] described an autosomal dominant developmental syndrome (MIM 617602) caused by germline heterozygous missense variants (NM_007313.2:c.734A > G p.(Tyr245Cys) and c.1066G > A p.(Ala356Thr)) in ABL1. Clinical features included congenital heart disease, skeletal malformations, dysmorphic facies, and failure to thrive. More recently, the clinical spectrum of the ABL1 malformation syndrome has been expanded to include hearing impairment, renal hypoplasia and ocular abnormalities [4].

Tyr245 lies in the SH2-kinase linker domain of ABL1 essential for the “docking” of the Src homology (SH3) domain in the inactive conformation of the protein [5]. Docking of SH3 to the SH2-kinase linker domain is one of three “linchpins” proposed to hold ABL1 in an inactive closed state, and therefore has an important auto-inhibitory role [6]. Phosphorylation of Tyr245 is necessary for maximal wild-type ABL1 kinase activity; a p.Tyr245Phe substitution reduces ABL1 kinase activity by 50% in vitro [7]. Unexpectedly, Wang et al. found that the c.734A > G p. (Tyr245Cys) variant increased ABL1 kinase activity [3], suggesting a possible gain-of-function effect.

Ala356 lies within the myristoyl-binding pocket of the ABL1 kinase domain. In isoform 1b (NM_007313) a myristoyl group bound to the N-terminal glycine of ABL1 occupies this pocket to stabilise the inactive conformation of the protein [8]. This docking of the myristoyl residue is the second “linchpin” of ABL1 auto-inhibition, which is lost in the BCR-ABL fusion product as the ABL1 N-terminus is truncated. The substitution of Ala356 for the polar amino acid threonine is expected to disrupt important hydrophobic interactions within the pocket. Indeed, the c.1066G > A p.(Ala356Thr) variant has increased kinase activity in vitro [3], consistent with a gain of function due to failure of auto-inhibition.

The developmental significance of ABL1 is illustrated by animal models. Abf2 mice harbour a targeted insertion-deletion in which exon 5 and part of exon 6 are replaced by the neomycin resistance gene [9]. Homozygotes die soon after birth with thymic and splenic atrophy, lymphopenia and osteoporosis [10]. Abf2m mice have a large targeted deletion of approximately one third of the ABL1 protein from the c-terminus [11]. Homozygotes have increased perinatal mortality, with defects of spleen, head and eye development [12]. Interestingly, Abf2m homozygotes of a C57BL/6J background also develop cardiac abnormalities [13]. Heterozygotes of both strains are largely unaffected, suggesting that ABL1 does not display haploinsufficiency, and supporting the possibility that the human germline missense variants act through a gain of function.

In this study, we have collected clinical and molecular details of six patients with deleterious ABL1 variants and have modelled the effects of these variants in vitro. We find that all but one of the variants identified in this cohort cluster in the myristoyl-binding pocket of ABL1, and that these variants increase the tyrosine kinase activity of ABL1 in vitro. These results are consistent with a gain-of-function effect, in which the variants disrupt the crucial auto-inhibitory binding of an N-terminal myristoyl group to its binding pocket. We find that variants in this myristoyl-binding pocket are a common cause of the ABL1 cardiac and skeletal malformation syndrome.

Subjects and methods

Patients

Patients 1, 3 and 5 were identified through genetic variant results returned via the Deciphering Developmental Disorders (DDD) study [14] (Complementary Analysis Project #278; DECIPHER IDs: patient 1 = 304716, patient 3 = 300146, patient 5 = 304918). UK ethical approval for the DDD study has been granted by the Cambridge South Research Ethics Committee (10/H0305/83). Patients 2, 4 and 6 were identified as part of routine clinical practice through clinical genetics services in Australia and New Zealand. Informed consent for publication was obtained for all patients whose clinical details and clinical photographs are included in this report. Ethical approval for the study involving patient 4 was obtained from the New Zealand Health and Disability Ethics Committee (16/STH/3). Clinicians of all patients reported to have ABL1 variants were contacted and requested to make assessments of variant pathogenicity in their patients.

Genetic analysis

For patients 1, 3 and 5, whole exome sequencing of saliva DNA samples was carried out through the DDD study. The DDD sequencing and bioinformatics framework has been previously described [15]. The DDD study identified 23 patients with missense variants in ABL1. Variants deemed pathogenic or likely pathogenic among those patients included in this report were confirmed by Sanger sequencing. The exome sequencing strategies used to identify the variants in patients 2 and 4 have been previously described [16, 17]. Each variant was confirmed by Sanger sequencing. Patient 6 underwent whole exome sequencing through Invitae (boosted
exome, proband only). Genomic DNA was enriched using a proprietary hybridisation-based protocol and sequenced on an Illumina platform. Sequences were aligned to GRCh37. Mean sequencing depth was 230x, with 99.9% of positions in reportable exons covered at ≥20x. Minimum calling depth was at least 20x. Targeted regions included at least 95% of the mappable exome, ±10 bp flanking regions. Promoters, untranslated and other non-coding regions were not interrogated. Variants were identified using a proprietary calling algorithm and confirmed by Sanger sequencing. Variants are annotated against GenBank transcript ID NM_007313.2. Exons are numbered as for GenBank accession NG_012034.1. The variants identified in this study have been submitted to the ClinVar database (accession numbers SCV001441170–SCV001441174).

**Plasmid mutagenesis**

A pCDNA3.1/V5-His A plasmid vector containing the ABL1 cDNA sequence was gifted by Yaping Yang, Baylor College of Medicine (Houston, TX, USA). Plasmid mutagenesis of the ABL1B transcript (NM_007313.2) was carried out for each variant following a modified version of the QuikChange Site-Directed Mutagenesis method (Agilent Technologies, Manchester, UK) using PfuUltra II Fusion HotStart DNA Polymerase (see Supplementary Information for details). Primers were designed using Agilent’s QuikChange Primer Design online tool (https://www.chem.agilent.com/store/primerDesignProgram.jsp) or using a partially overlapping primer design [18] (see Supplementary Information for primer sequences). Mutagenised plasmids were used to transform One Shot TOP10 Chemically Competent E. coli (Thermo Fisher Scientific, Paisley, UK). Individual clones were isolated and ABL1 fully sequenced to confirm the correct sequence and presence of the required variant.

**Transfection and ABL1 activity assay**

To investigate ABL1 kinase activity in vitro, HEK293T cells were transfected with plasmid constructs encoding wild-type or variant ABL1 cDNA using Lipofectamine 2000 (Thermo Fisher Scientific, Paisley, UK). After 48 h, cells were serum starved for 1 h before preparation of protein lysates. Phosphorylation of ABL1 and the ABL1-specific substrate STAT5B was measured by western blotting using the following antibodies: ABL1 (clone OP20) (EMD Millipore, Billerica, MA, USA); Phospho-ABL1 (Tyr245) (Cell Signaling Technology, Danvers, MA, USA; #2861); Phospho-ABL1 (Tyr241) (Abcam, Cambridge, UK), STAT5 (Cell Signaling Technology, #9363); phosho-STAT5 (Cell Signaling Technology, #9359); Phosphotyrosine (Fisher Scientific, PY20), Actin (Santa Cruz Biotechnology, Dallas, Texas, USA; SC-10731). Imatinib was purchased from Stratech (Ely, UK). To assess effect on phosphorylation activity, 1 µM imatinib was added during the 1 h serum starvation before lysis.

**Analysis of variation and conservation in ABL1**

Non-pathogenic missense variants of ABL1 in patients with abnormal phenotypes were collated from the DECIPHER database [14]. ABL1 missense variants in healthy population controls were identified through the gnomAD [19] and EVS [20] databases. PhyloP basewise evolutionary conservation scores [21] for every position in the transcript (NM_007313.2) were obtained through the UCSC table browser [22]. Missense constraint scores for every codon were obtained from the MTR Gene Viewer [23]. Pathogenic variants were analysed by the standard pathogenicity prediction programs PolyPhen [24], SIFT [25], MutationTaster [26], and CADD [27].

**Results**

**Clinical features**

The clinical features of our cohort are summarised in Table 1, and representative clinical photographs are given in Fig. 1. All six individuals recapitulate the phenotype of congenital heart disease, skeletal malformations and characteristic facies which had been previously described. Hearing impairment has recently been identified as a common feature of the ABL1 malformation syndrome [4]; four of our cohort exhibit conductive or mixed conductive/sensorineural hearing impairment, which was severe and persistent in one patient. Interestingly, two individuals have tall stature, in contrast to short stature in the majority of cases. Some other phenotypic features are also over-represented in our cohort, including camptodactyly (5/6) and microcephaly (5/6). Others are under-represented or absent, including pectus deformity (1/6), ear abnormalities (1/6), gastro-intestinal disorders (1/6), joint hyper-extensibility (0/6), dental decay (0/6), and genito-urinary disorders (1/6) (Table S1).

The most common clinical features across all described individuals with ABL1 variants are dysmorphic facies (18/18), finger/toe abnormalities (17/18), congenital heart disease (14/18), failure to thrive (14/18), developmental delay (11/18), IUGR (10/18), ear abnormalities (9/18), palatal deformity (9/18) and microcephaly (9/18) (Table S1).

**Pathogenic ABL1 variants**

We identified six individuals with five deleterious de novo missense variants in ABL1. Four of these variants have not
| General | Patient | 1 | 2 | 3 | 4 | 5 | 6 |
|---------|---------|---|---|---|---|---|---|
| Variant | c.1066G>A; p. (Ala356Thr) | c.1066G>A; p. (Ala356Thr) | c.1354G>A; p. (Ala452Thr) | c.1574T>C; p. (Val525Ala) | c.1582G>A; p. (Glu528Lys) | c.731T>C; p. (Val244Ala) |
| General | Age (years) | 4 | 29 | 13 | 6 | 40 | 37 |
| Gender | Female | Female | Male | Female | Male | Male |
| Growth | Age at measurement (years) | 3.5 | 25 | 13 | 6 | 40 | 36 |
| Height/Length (cm) | 92.4 (<9th centile) | 150.8 (<0.4th centile) | 170.9 (98th centile) | 109 (9th centile) | 191 (<99.6th centile) | 188 (98th centile) |
| Weight (kg) | 11.6 (0.4th centile) | 37.6 | 56.3 (91st centile) | 45 (2nd centile) | 82.5 | 103 |
| Head circumference (cm) | 46 (<0.4th centile) | 51 (<3rd centile) | 52.1 (2nd centile) | 45 (<2nd centile) | 52.6 (<<0.4th centile) | 60.5 (90th centile) |
| Intratrophic growth restriction | Yes (BW 2.2kg at 37 weeks) | Yes (BW 2.41kg at 36 weeks) | No (BW 2.75kg at 37 weeks) | Yes (mild) | No (BW 3.09kg at 41 weeks) | Yes |
| Growth failure | Yes | Yes | Yes | Yes | Yes | Yes |
| Feeding difficulties | Short stature | Short stature | Tall stature | Short stature (mild) | Tall stature (proportionate) | Tall stature |
| Development | Developmental delay | High-arched eyebrows, full cheeks | Elongated face, narrow maxilla, facial asymmetry, scaphocephaly | Moderate (global) | Mild | Mild | Mild (mainly motor) |
| Dysmorphic features | Face | High-arched eyebrows, full cheeks | Elongated face, narrow maxilla, facial asymmetry, scaphocephaly | Moderate (global) | Mild | Mild | Mild (mainly motor) |
| Eyes | Deep-set eyes | Almond-shaped eyes, epibulbar pharion | Epicanthic folds | Ptosis, proptosis | Prominent ears, lobeless ears | Prominent nasal bridge, low columella |
| Ears | Asymmetry of the ears | Prominent ears, lobeless ears |
| Nose | Hypoplastic alae nasi | Long narrow nose, hypoplastic alae nasi | Prominent nasal tip, broad nasal root | Prominent nasal bridge, low columella | Long narrow nose |
| Mouth | Thin lips, dental crowding | Small downturned mouth | Down-turned mouth |
| Palate | High-arched palate | Small pointed chin | Small pointed chin | High palate |
| Chin | Microretrognathia | No | Yes | Yes |
| Cardiovascular | Atrial septal defect | No | Yes | Yes | Yes |
| Venticular septal defect | No | Yes | Yes | Yes |
| Aortic root dilatation | No | Yes (mild) | Yes (mild) | Yes (mild) |
| Other | Supra-valvular pulmonary stenosis | Patent ductus arteriosus | Bicuspid aortic valve, pacemaker for intermittent junctional rhythm | Idiopathic hypertension, mild concentric left ventricular hypertrophy |
| General Variant | Patient | 1 | 2 | 3 | 4 | 5 | 6 |
|-----------------|---------|---|---|---|---|---|---|
| c.1066G>A; p. (Ala356Thr) | c.1066G>A; p. (Ala356Thr) | c.1354G>A; p. (Ala452Thr) | c.1574T>C; p. (Val525Ala) | c.1582G>A; p. (Glu528Lys) | c.731T>C; p. (Val244Ala) |
| Skeletal Pectus excavatum | Yes | Yes (surgical intervention) | Yes | Yes (thoracic) | |
| Scoliosis | | | | | |
| Finger/toe abnormality Camptodactyly of fingers, arachnodactyly | Camptodactyly of fingers | 2–3 toe syndactyly, camptodactyly of fingers, clinodactyly of 5th fingers | 2–3 toe syndactyly, clinodactyly of 4th and 5th fingers, tapered fingers | Camptodactyly, bilateral Dupuytren’s contracture, slender fingers | Camptodactyly, clinodactyly of 5th finger, slender fingers |
| Foot deformity | | Metatarsus adductus | | | |
| Other | Hypoplasia of right lower limb | | | | |
| Joints Hypermobility | None | Mild elbow laxity | Joint laxity (Beighton score 4/9) | None | None |
| Other | | | | | Joint swelling of fingers, osteoarthritis of hips |
| Gastrointestinal Constipation/Reflux | No | Constipation | No | No | No |
| Genito-urinary Renal tract | No | Left renal agenesis | No | No | Micropenis, hydrocoele |
| Reproductive tract Absent left vas deferens | | | | | |
| Skin Thin skin | Yes | Yes | Yes | | |
| Other Fibromata of hands and feet | | | | | Cutis marmorata |
| Other Hearing impairment Congenital conductive hearing impairment | Chronic otitis media, secondary conductive hearing loss | Mixed conductive/sensorineural hearing impairment (50dB loss bilaterally) | Mixed conductive/sensorineural hearing impairment | | |
| Other | Lacrimal duct stenosis, recurrent pneumothorax | Bilateral inguinal hernias, fetal choroid plexus cysts, spontaneous pneumothorax | Varicose veins, liver cirrhosis | Oligodontia, distal upper limb weakness, prominent veins | |

Adult head circumference centiles are based on charts produced by Bushby et al [34].

BW birth weight.
been previously described. Our findings are summarised in Fig. 2. Four of the five variants cluster in the myristoyl-biding pocket within the kinase domain, which is a critical auto-inhibitory region in ABL1 (Fig. 3).

The molecular characteristics of each variant are shown in Table 2. The nucleotide and amino acid at each position is highly conserved between species. All variants are predicted to be deleterious by multiple pathogenicity prediction programs. None of the variants are present in gnomAD. All novel variants were classified as “pathogenic” or “likely pathogenic” by ACMG criteria [28].

Benign ABL1 variation

Whereas pathogenic heterozygous germline ABL1 variants cluster within and adjacent to the ABL1 kinase domain, non-pathogenic ABL1 variants in the DECIPHER cohort largely lie outside this region (Fig. 2a). Benign germline variation among gnomAD participants is found in every domain of ABL1 but is relatively scarce within the kinase domain (Fig. 2a). Mean PhyloP scores are significantly higher in the kinase domain and the SH3/2 domains than the rest of the transcript, while MTR scores are correspondingly lower in these regions (Fig. 2b, Supplementary Table 2). Codons and individual bases within the kinase domain are therefore prone to greater missense constraint and evolutionary conservation than other positions in the transcript. Somatic ABL1 missense variants associated with imatinib resistance in BCR-ABL leukaemias cluster exclusively within the kinase domain (Fig. 2a). One residue (Ala452) is associated both with pathogenic variation in the germline, and imatinib resistance as a somatic variant.

In vitro ABL1 assay

To investigate ABL1 kinase activity in vitro, HEK293T cells were transfected with plasmid constructs encoding wild-type or variant ABL1 cDNA. Cell lysates were assayed for phosphorylation of ABL1-specific substrates by immunoblotting (Fig. 4). Phosphorylation of ABL1-Tyr245 and STAT5B were substantially increased in lysates transfected with the c.1066G > A p.(Ala356Thr) construct, consistent with previous reports [3]. Phosphorylated ABL1 and STAT5B were also increased for the c.1354G > A p.(Ala452Thr), c.1574T > C p.(Val525Ala), and c.1582G > A p.(Glu528Lys) constructs (Fig. 4a). These results are consistent with gain of ABL1 tyrosine kinase activity due to loss of auto-inhibition by myristoyl binding.

No evidence of ABL1 activation was seen for the negative control, c.881A > G p.(Glu294Gly). This variant was identified through the DDD study in a patient with a likely pathogenic de novo variant in another gene.

The c.731T > C p.(Val244Ala) construct caused an increase in phosphorylation of STAT5B and of overall tyrosine phosphorylation, with reduced autophosphorylation at Tyr245 compared to wild-type. Alteration of Val244 to alanine therefore appears to result in loss of phosphorylation at this site [7]. However, the previously described pathogenic c.734A > G p.(Tyr245Cys) variant also abolishes phosphorylation at this site [3]. Furthermore, other variants within the SH2-catalytic domain linker region have previously been shown to cause ABL1 to adopt an active conformation by disrupting the inhibitory interaction between the SH3 and catalytic domains [7, 29].
A distinctive feature of the variants we describe is their close spatial relationship to one another in the three-dimensional crystal structure of the protein, and specifically their position within the myristoyl-binding pocket. This close spatial relationship is not immediately apparent from the position of these variants in the ABL1 transcript, and yet suggests a self-evident mechanism by which they can exert a deleterious gain-of-function effect. We predict that other amino-acid substitutions within the myristoyl-binding pocket, particularly those which disrupt hydrophobic interactions or introduce bulky amino acids, will also be deleterious in the germline. We also predict that missense variation or in-frame deletion of the N-terminal glycine of ABL1, which carries the myristoyl modification, will be deleterious.

We also describe a novel c.731T→C p.(Val244Ala) variant which lies in the SH2-kinase linker domain, immediately adjacent to a previously described c.734A→G p.(Tyr245Cys) variant. Other variants in this linker domain are known to disrupt the inhibitory docking of the SH3 domain to the SH2-kinase linker domain, and thereby constitutively activate the ABL1 kinase [7]. Notably, the c.731T→C p.(Val244Ala) variant we describe causes reduced phosphorylation of ABL1-Tyr245 in vitro. Phosphorylation of Tyr245 is necessary for maximal activation of the wild-type ABL1 [7], yet the c.731T→C p.(Val244Ala) and c.734A→G p.(Tyr245Cys) variants must activate ABL1 independently of the Tyr245 phosphorylation status. Recently, an in-frame deletion of c.434_436del p.(Ser145del) has also been associated with the ABL1 developmental syndrome [30], but no functional work has yet been performed to characterise the effect of this variant on ABL1 kinase activity.

ABL1 is best known as a proto-oncogene. In CML and other haematological malignancies, a somatic translocation between chromosomes 9 and 22 produces the Philadelphia chromosome [31], carrying a BCR-ABL fusion gene. As the N-terminus of ABL1 is lost in the fusion product, the auto-inhibitory binding of the myristoyl group to the kinase domain is abolished, and ABL1 gains constitutive tyrosine kinase activity which drives cellular proliferation [32].

Tyrosine kinase inhibitors (TKIs) specific to ABL1, such as imatinib, are the mainstay of treatment for CML. However, resistance to TKI therapy is strongly associated with somatic missense variants in the ABL1 kinase domain, particularly in the ATP-binding loop (P loop) and at TKI-specific binding sites [33].

It is noteworthy that none of the activating germline variants we describe have been associated with somatic TKI resistance. If, as we argue, germline variants in the kinase domain sterically hinder myristoyl binding, they will functionally mimic the loss of the ABL1 N-terminus in BCR-ABL. We therefore expect that TKIs effective against BCR-
ABL should be similarly effective against these variant proteins. Indeed, TKIs may potentially in some way be therapeutically beneficial for the ABL1 developmental syndrome or its complications, for example in limiting aortic root dilatation or reducing the tendency for dilatation to occur. However, given that this condition appears to affect embryonic and fetal development, any more complete therapeutic effect would require as early treatment as possible and entail long-term therapy, potentially risking adverse drug effects. While imatinib is used to treat paediatric CML cases, it is expected to be teratogenic in pregnancy. Further work is therefore required to ascertain whether therapeutic scope exists for use of imatinib or similar TKIs in this condition.

It is also noteworthy that activating somatic missense variants in ABL1 have not been found to independently cause haematological malignancy, although both isoforms of ABL1 are ubiquitously expressed. Disruption of myristoyl binding alone may not activate ABL1 sufficiently to drive malignancy. Two other “linchpins” of ABL1 autoinhibition (the docking of the SH3 domain to a polyproline helix in the SH2-kinase linker, and an N-terminal “brace” over the SH3-SH2 unit) may prevent its excessive activation [6]. Indeed, both the N-terminal “brace” and the myristoyl group are lost in the BCR-ABL fusion. It is not clear whether the activating germline variants we describe can act as driver variants in malignancy. No patients with the ABL1 skeletal and cardiac malformation syndrome described here or elsewhere are reported to have haematological malignancy, but longitudinal follow up of these patients will be required to better determine this.

From a clinical perspective, we believe this condition to be a phenotypically distinctive and recognisable syndrome based on affected individuals’ dysmorphology and associated clinical features. Phenotypes such as skeletal malformations, aortic root dilatation and pneumothorax point towards an overlap with genetic connective tissue disorders and we recommend that ABL1 be borne in mind in such cases. The high prevalence of hearing impairment makes audiological assessment advisable. Furthermore, assessment of the aortic root diameter at the time of diagnosis may also be appropriate in individuals found to have pathogenic ABL1 variants. Evidence is currently lacking as to the true risk of aortic aneurysm and dissection in this condition. We are not aware of any affected individuals having had rapid progressive aortic dilatation requiring surgical intervention and this may therefore suggest a more indolent course. However, a precautionary approach of ongoing aortic root screening similar to that used for Marfan syndrome may be appropriate until such time as more accurate natural history data are available.

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### Table 2: Molecular details of deleterious ABL1 missense variants.

| General          | Patient | 1             | 2             | 3             | 4             | 5             | 6             |
|------------------|---------|---------------|---------------|---------------|---------------|---------------|---------------|
| Molecular labels | Position (hg19/GRCh37) | 9:133748348 | 9:133748348 | 9:133753828 | 9:133755890 | 9:133755898 | 9:133738274 |
| Exon Number      |         | 6             | 6             | 8             | 10            | 10            | 4             |
| Transcript (RefSeq) |         | NM_007313.2   | NM_007313.2   | NM_007313.2   | NM_007313.2   | NM_007313.2   | NM_007313.2   |
| c.              |         | c.1066G > A   | c.1066G > A   | c.1354G > A   | c.1574T > C   | c.1582G > A   | c.731T > C   |
| p.              |         | p.(Ala356Thr) | p.(Ala356Thr) | p.(Ala452Thr) | p.(Val525Ala) | p.(Glu528Lys) | p.(Val244Ala) |
| Conservation     |         | Highly conserved | Highly conserved | Highly conserved | Highly conserved | Highly conserved | Highly conserved |
| Nucleotide (phyloP) |         | 6.067         | 6.067         | 4.161         | 4.998         | 6.049         | 4.736         |
| Amino Acid Conservation |         | D. melanogaster | D. melanogaster | C. elegans | X. tropicalis | D. rerio | F. catus |
| Pathogenicity    |         | ExAC Allele Frequency | 0.846 | 0.846 | 0.657 | 0.584 | 0.583 | 0.6 |
|                  |         | SIFT          | Damaging      | Damaging      | Damaging      | Damaging      | Damaging      |
|                  |         | 0.019         | 0.019         | 0.013         | 0.007         | 0.048         | 0.02 |
|                  |         | Mutation Taster | Disease causing | Disease causing | Disease causing | Disease causing | Disease causing |
|                  |         | P: 0.999      | P: 0.999      | P: 0.999      | P: 0.999      | P: 0.999      | P: 0.998      |
|                  |         | CADD          | 31            | 31            | 27            | 28.5          | 33            | 27.3 |
|                  |         | ACMG Classification | Pathogenic | Pathogenic | Likely Pathogenic | Likely pathogenic | Likely pathogenic | Uncertain |
|                  |         | Inheritance   | De novo       | De novo       | De novo       | De novo       | De novo       | Unknown |
|                  |         | Zygosity      | Heterozygous  | Heterozygous  | Heterozygous  | Heterozygous  | Heterozygous  | Heterozygous |

*The variant in patient 6 would be classified as “Likely pathogenic” if PP4 were applied (highly specific phenotype for a disease with a single genetic aetiology) or “Pathogenic” if PS3 were applied in light of the experimental findings in this paper (functional studies supportive of a damaging effect).*
Fig. 4 Missense variants cause increased ABL1 tyrosine kinase activity in vitro. a Tyrosine kinase activity of ABL1 missense constructs. Missense variants NM_007313.2:c.1066G > A p.(Ala356Thr), c.1354G > A p.(Ala452Thr), c.1574T > C p.(Val525Ala), and c.1582G > A p.(Glu528Lys) markedly increase the phosphorylation of ABL1 at residue Tyr245, and the phosphorylation of the ABL1-specific substrate STAT5B, compared to wild-type. The c.881A > G p.(Glu294Gly) construct (for which the variant is not thought to be deleterious), does not increase phosphorylation of ABL1 or STAT5B. b Treatment with 1 µM imatinib results in complete loss of phosphorylation activity.

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