Acute lymphoblastic leukemia in a child with Leri-Weill syndrome and complete SHOX gene deletion: A Case Report

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Background. Leri-Weill syndrome (LWS) ranks among conditions with short stature homeobox gene *(SHOX)* haploinsufficiency. Data on possible association of *SHOX* aberrations with malignant diseases are scarce.

Methods and Results. We report a unique case of an 8-year-old girl who was successfully treated for acute lymphoblastic leukemia (pre-B ALL, intermediate risk) and was subsequently diagnosed with LWS due to characteristic clinical appearance (short disproportionate stature, Madelung deformity of the wrist) and molecular genetic examination (complete deletion of *SHOX*). An identical *SHOX* deletion was identified also in the patient's mother. Leukemic cells of the patient were retrospectively examined by array comparative genomic hybridization (aCGH), which revealed five regions of deletions at chromosome X, including the *SHOX* gene locus.

Conclusion. Growth retardation in children with hemato-oncologic malignancies cannot always be attributed to cytotoxic treatment and should be carefully evaluated, especially with regards to growth hormone therapy.

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INTRODUCTION

Leri-Weill syndrome or Léri–Weill dyschondrosteosis (LWS), first described in 1929 (ref.[1]), is a rare pseudoautosomal dominant genetic disorder characterized by skeletal dysplasia with disproportionate short stature, mesomelia (i.e. shortening of the middle parts of the limbs) and characteristic Madelung wrist deformity[2]. LWS is caused by mutations of the short stature homeobox (*SHOX*) gene located within the pseudoautosomal region (PAR1) of both sex chromosomes (Xp22 and Yp11). *SHOX* haploinsufficiency was reported as causative for short stature in women with Turner syndrome[3] and subsequently its mutations have been identified in patients with LWS and Langer mesomelic dysplasia[4,5] and in approximately 2-16% of cases with idiopathic (nonsyndromic) short stature including children born small for gestational age[3,6-9]. *SHOX* abnormalities have been associated with various physical features such as scoliosis, increased carrying angle of the elbow, deformities of the forearm and tibiae, shortening of the fourth and fifth metacarpals, malformations of the middle ear, exostoses, high-arched palate, micrognathia and muscular hypertrophy[8]. Mutations of other genes located within Xp22.3 cause monogenic X-linked syndromes such as Kallmann syndrome, chondrodysplasia punctata, ichthyosis and learning/behavioral difficulties[10,11].

Much attention has been paid to *SHOX* as one of the main growth regulators, however, less is known about its association with other pathological conditions, such as disorders of hematopoiesis and malignant tumors. Here we report a unique case of coincidence of LWS and acute lymphoblastic leukemia (ALL).

CASE REPORT

An 8-year-old female was diagnosed with ALL (L2 morphology, pre-B immunophenotype, prednisone good response, classified into the intermediate risk group). The karyotype of the leukemic cells was 46,XX,del(X)(Xp22.33--Xp21.3::Xp11.1--Xq22.2::Xqter),t(12;21)(p13;q22)[15]/46,XX[6]. Fluorescence in situ hybridization (FISH) using SHOX region probe (MD Short Stature (Xp22)/SE X (Kreatech, Amsterdam, Netherlands) showed two unrearranged signals with both Xp and centromeric regions retained; however, five regions of deletion comprising the *SHOX* gene locus and one region of loss of heterozygosity (LOH) were detected by array comparative genomic hybridization (aCGH, SurePrint G3 Human CGH+SNP Platform, 4x180K, data analysis CytoGenomic software, both Agilent, Santa Clara, CA, USA). Furthermore, deletion in 3p24.1, 7p14.3 and mono-
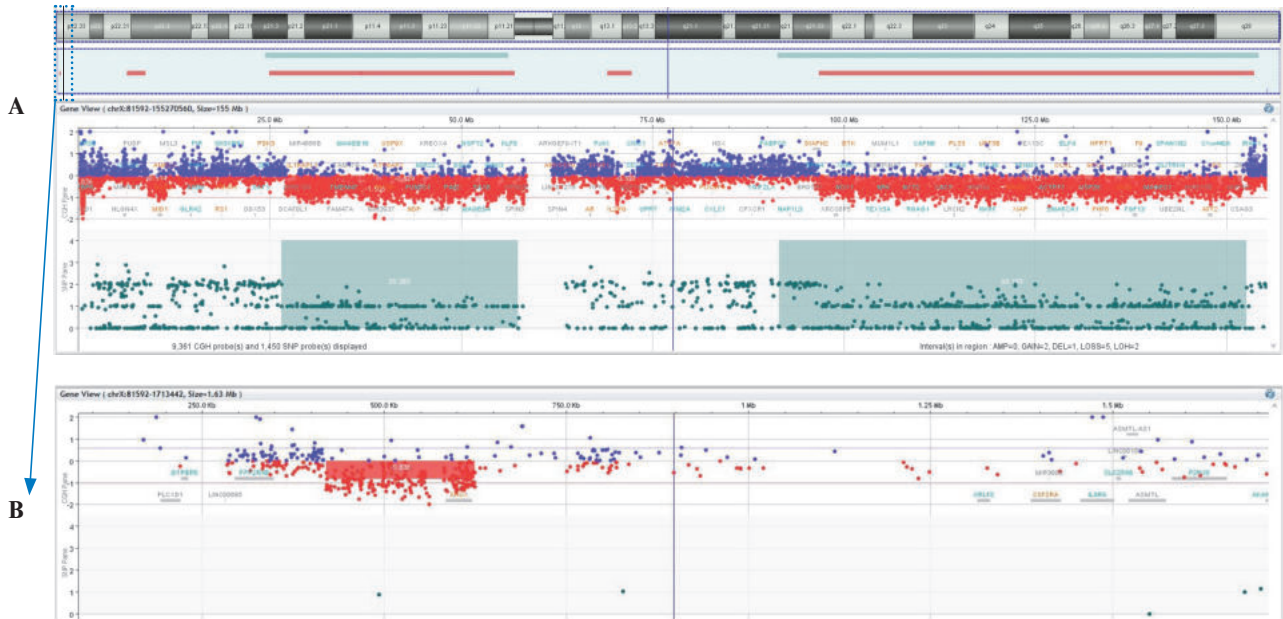
Fig. 1. Chromosome X in leukemic cells of patient with Leri-Weill syndrome. A) Ideogram and aCGH log2 ratio plot of chromosome X displaying 5 regions of deletion (red lines) at Xp22.33, Xp22.31p22.2, Xp21.3p11.1, Xq13.1q13.2 and Xq21.33q28 (CGH Pane) and SNP array result showing the number of uncut alleles and LOH region Xq21.31q21.33 (grey line, SNP Pane). B) Detail of the Xp22.33 region (81592-1713442).

and biallelic deletions involving the retinoblastoma gene (*RB1*) on chromosome 13q were present. The result of aCGH in leukemic cells was
arr[hg19] 3p24.1(27139147_29312423)x1,
7p14.3(30821635_31233233)x1,
13q12.3q14.3(31729646_53436659)x1,
13q14.2(48985982_49150225)x0,
Xp22.33(420237_624896)x1,
Xp22.31p22.2(9053476_11401244)x1,
Xp21.3p11.1(27034198_58188680)x1,
Xq13.1q13.2(69960578_73063439)x1,
Xq21.31q21.33(87981878_96791827)x2 hmz,
Xq21.33q28(96829124_152053777)x1 (Fig. 1).
The list of involved genes is given in Table 1. The aCGH datasets are available from the NCBI Gene Expression Omnibus (GEO) repository: http://www.ncbi.nlm.nih. gov/geo/query/acc.cgi?acc=GSE110235. The patient was successfully treated at our department with the ALL-BFM 95 protocol. She is alive in first complete remission and has experienced no long term sequelae to the ALL therapy.

Nevertheless, at the regular follow-up examination at the age of 11 years, disproportionate short stature with shortening of the forearms and tibia and muscular hypertrophy were recognized (Fig. 2). The patient's height at the age of 11 years was 127 cm (-2.5 standard deviation (SD), Fig. 3). On wrist X-ray, the skeletal age was accelerated by 1 year and Madelung deformity was detected (Fig. 4).

The patient's history was reviewed: she was delivered at term with a normal birth weight (3000 g) and birth length (52 cm). Her psychomotor development was normal. However, her growth curve since early childhood was following the approximately -2.5 SD percentile of the
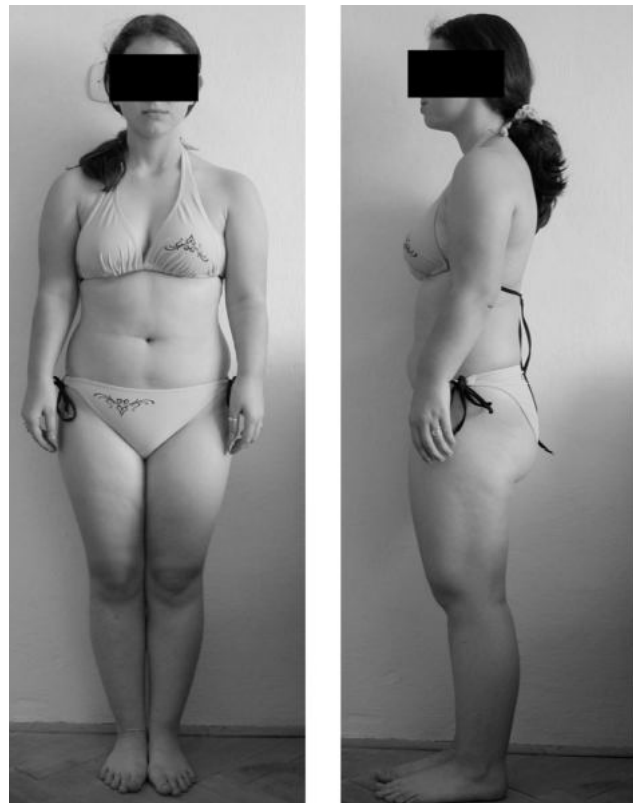

Fig. 2. Short disproportionate stature in a patient with Leri-Weill syndrome and ALL. The patient had an adult height of 147.7 cm (picture taken at the age of 15 years). Note shortening of forearms and tibia and muscular hypertrophy.
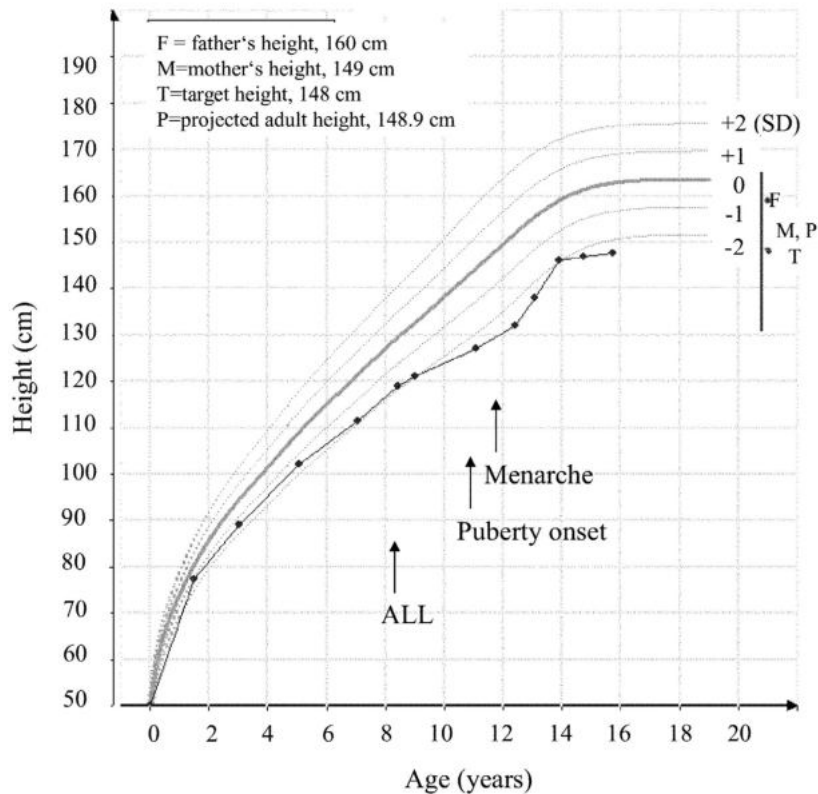
Fig. 3. Growth curve of patient with Leri-Weill syndrome and ALL. Growth charts by Freeman et al., 1995. F, father's height; M, mother's height; T, target (estimated) height.

national height standards. This had been previously attributed to a familial disposition; the patient's parents also had short stature: mother 149.2 cm (-2.6 SD), maternal grandmother 150 cm and father 160 cm (-2.4 SD, Fig. 3).

Mutations and deletions in the *SHOX* gene were examined in peripheral blood using polymerase chain reaction (PCR), heteroduplex formation, single nucleotide polymorphism (SNP)-based detection by denaturing high-pressure liquid chromatography (DHPLC) and sequencing (SHOX-DNA-Dx, Esoterix, CA, USA; www.esoterix.com). Complete deletion of the *SHOX* gene was confirmed. Despite the fact that there was an ongoing clinical trial with growth hormone in 2001, the patient did not receive the treatment due to late diagnosis. She had an early onset of puberty (at 11 years) and at the time of LWS diagnosis her Tanner stages were B2, Ph1, A1. The patient had menarche at the age of 11 years and 10 months and her postmenarchal growth was minimal; her final height was 147.7 cm, i.e. approximately -2.5 SD (Fig. 3). She delivered a boy at the age of 19 years.

The suspicion of LWS was raised in the patient's mother who also displayed disproportionate short stature and Madelung deformity. Using the same molecular genetic test (SHOX-DNA-Dx, Esoterix), the identical complete deletion of the *SHOX* gene was confirmed.

The patient's parents provided informed consent for publication.

DISCUSSION

The list of indications for growth hormone therapy in children is increasing, with LWS being added in 2007 in the European Union. Thus, an appropriate detection of *SHOX* aberrations is vital for timely initiation of growth hormone treatment. Reports on *SHOX* status in malignant diseases are scarce. A connection between *SHOX2* methylation of cell-free circulating DNA and lung cancer has been suggested; however *SHOX2*, unlike *SHOX*, is an autosomal homeobox gene located on chromosome 3 (ref.[12,13]). Translocations of the *TFE3* transcription factor gene at Xp11 in tumor cells are related to papillary renal cell carcinoma[14]. Regarding disorders of hematopoiesis, a predisposing locus for Hodgkin lymphoma was placed in proximity to *SHOX* in PAR1. This was based on familiar coinheritance of LWS and Hodgkin lymphoma and a predominance of same-sex sibling pairs among those affected with Hodgkin disease[15]. Recurrent somatic mutations in *P2RY8* gene located in PAR1 have been identified in cases of diffuse large B-cell lymphoma[16]. In an elderly male with concurrent LWS, Klinefelter syndrome and hepatocellular carcinoma followed by myelodysplastic syndrome, breakpoint regions did not involve cancer genes and thus the causality between dic(X;Y)(p22.33;p11.32) and mentioned malignancies was considered unlikely[17].

PAR1 includes genes with an important role in immune response and hematopoiesis, such as granulocyte macrophage colony-stimulating factor receptor (*CSF2RA* or *GM-CSFR*), interleukin 3 receptor alpha (*IL3RA*)
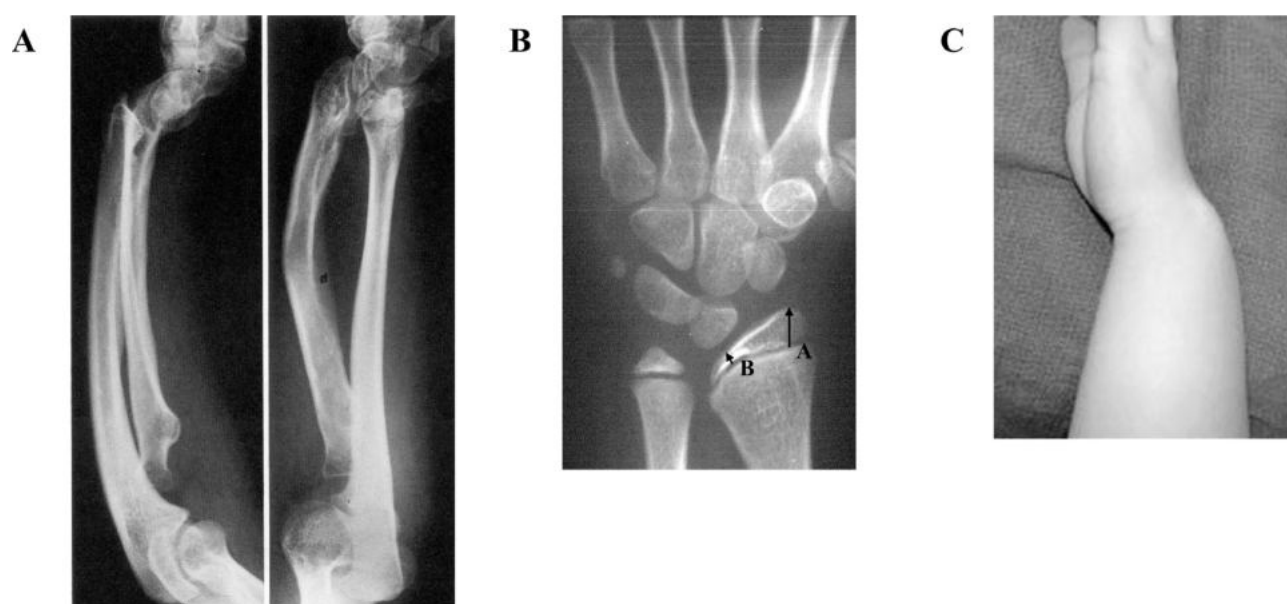
Fig. 4. Madelung deformity of the wrist. The deformity is congenital, bilateral and is characterized by growth disturbance in the volar-ulnar distal radial physis. This results in a bowing and shortening of radius and dorsal dislocation of distal ulna (A). (B) Triangulation of distal radial epiphysis with shortening of the ulnar segment (A/B index>4.0). (C) Volar translation of the hand and wrist causes restriction of movement.

Table 1. Selected genes and biological processes affected by gene deletions in leukemic cells of the patient with LWS and ALL.

GO_ID	Biological Process	Total Mapped Genes	Found Genes	Fraction Found/Total	Gene List
GO:0006281	DNA repair	516	12	0.02	APEX2,BRCA2,CETN2,CUL4B,HUWE1,MORF4L2,NONO,PDS5B,RFC3,RPA4,SMC1A,UBE2A
GO:0060215	Primitive hemopoiesis	9	1	0.11	GATA1
GO:1903708	Positive regulation of hemopoiesis	163	10	0.06	ATP11C,BTK,FOXP3,GATA1,N4BP2L2,NKAP,OGT,RB1,SASH3,TNFSF11
GO:0060216	Definitive hemopoiesis	21	1	0.05	GATA1
GO:0035162	Embryonic hemopoiesis	22	1	0.05	GATA1
GO:1903706	Regulation of hemopoiesis	326	12	0.04	ATP11C,BTK,FOXP3,GATA1,HMGB3,N4BP2L2,NKAP,OGT,RB1,SASH3,TFE3,TNFSF11
GO:0030097	Hemopoiesis	734	20	0.03	ALAS2,ARL11,ATP11C,AZI2,BRCA2,BTK,CD40LG,EBP,EOMES,FOXP3,GATA1,GPC3,HMGB3,N4BP2L,NKAP,OGT,RB1,SASH3,TFE3,TNFSF11
GO:1903707	Negative regulation of hemopoiesis	131	3	0.02	FOXP3,HMGB3,N4BP2L2
GO:0035603	Fibroblast growth factor receptor signaling pathway	1	0	0	
GO:0040008	Regulation of growth	641	19	0,03	AGTR2,DDX3X,ENOX2,FGF13,FHL1,GHRHR,GPC3,MCTS1,MORF4L2,MTM1,NRK,PSMD10,RB1,SASH3,SPG20,TRO,TRPC5,TSPYL2,WWC3

and cytokine receptor-like factor 2 (*CRLF2)* whose rearragements resulting from deletions in PAR1 have been detected in leukemic cells in a substantial percentage (14%) of children with ALL and reported to be associated with mutation of JAK kinases, *IKZF1* alterations and poor treatment outcomes[18]. Adverse prognostic impact of *CRLF2* rearrangements leading to *CRLF2* overexpression in children and adults with ALL has since then been confirmed by numerous studies[19,20].

The *CRLF2* gene in leukemic cells of our patient taken at the time of ALL diagnosis was undeleted, as well as *P2RY8, CSF2RA* and *IL3RA*. False negativity of FISH for *SHOX* deletion was caused by the fact that the probe only partially covered the *SHOX* gene and fluorescence was emitted from the residual part of the probe. Monoallelic *SHOX* deletion was confirmed by aCGH and thus the phenotype of the leukemic blasts was in this case in accordance with genotype.

Among 1184 gene loci within the deleted regions, genes involved in DNA repair, hematopoiesis and growth regulation were identified in our patient (Table 1), most importantly *ARL11, BRCA2, FOXO4* and *GATA1* which are associated with leukemia[21-23].

The clinical significance of 13q deletion involving the retinoblastoma gene (*RB1*), a known tumor supressor gene whose numerous mutations are associated with retinoblastoma, several subtypes of sarcoma, melanoma and other types of cancer, is unknown in our patient. The role of loss or inactivation of *RB1* in leukemogenesis has been documented[24].

Regarding muscular hypertrophy in our patient, we also considered that the involvement of the dystrophin gene (*DMD*, Xp21.2-p21.1) within the deleted Xp region may be the causal aberration. However, the *DMD* deletion was found only in leukemic cells and information on germinal *DMD* status is missing. Furthermore, muscular hypertrophy, particularly involving calf muscles, affects up to 80% of the LWS patients[8,25], which is much more frequent than in molecular dystrophy carriers. No other confirmed cases or signs of muscular dystrophy have been observed in the patient or her family members. Therefore, we assume that in this case the association of calf hypertrophy with *DMD* gene is unlikely.

Unfortunately, the patient was later lost to follow-up, and therefore the remission sample was unavailable for aCGH examination. An absence of somatic DNA for further analyses, such as aCGH and next generation sequencing, is a significant drawback of our study.

The case presented here of concurrent LWS and ALL is the first published to date. A previously reported case of a child with thrombocytopenia and absent radii (TAR syndrome) who developed ALL (ref.[26]) is relevant; although genetically distinct, TAR syndrome similarly manifests with radial growth abnormalities and short stature. It is therefore necessary to distinguish between growth retardation as a side effect of ALL treatment, which is infrequent[27], and possibly other unrelated pathological conditions. We emphasize the importance of careful evaluation of auxology and somatic morphology for timely diagnosis of *SHOX* haploinsufficiency in children with

unexplained short stature. Precise detection of *SHOX* abnormalities is important not only for therapeutic intervention but also for the estimation of prognosis, genetic counseling and search for other affected family members.

CONCLUSION

This is a unique report of a child diagnosed with inherited Leri-Weill syndrome (LWS) with characteristic clinical features and complete deletion of the *SHOX* gene in the first remission of acute lymphoblastic leukemia (ALL) at the age of 11 years. We highlight the importance of auxology and somatic morphology assessment in children with malignant disease. Growth retardation can be caused not only by chemo- or radiotherapy but also by other diseases or rare underlying syndromes in which timely treatment with growth hormone is indicated.

Author contributions: VM, MH and MSH: study design and supervision; JV: data collection, literature search and manuscript writing; JZ, EK and VM: clinical management; MJ, HU: cytogenetics including database record; PV, MH: data analysis, gene search. All co-authors have read the final manuscript within their respective areas of expertise and participated sufficiently in the study to take responsibility for it and accept its conclusions.
Conflicts of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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