Prospective Study of Epigenetic Alterations Responsible for Isolated Hemihyperplasia/Hemihypoplasia and Their Association With Leg Length Discrepancy

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Research

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Abstract

Background: Hemihyperplasia and hemihypoplasia result in leg length discrepancy (LLD) by causing skeletal asymmetry. Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) are opposite growth-affecting disorders caused by opposite epigenetic alterations at the same chromosomal locus, 11p15, to induce hemihyperplasia and hemihypoplasia, respectively. Because of their somatic mosaicism, BWS and SRS show a wide spectrum of clinical phenotypes. We evaluated the underlying epigenetic alterations and potential epigenotype-phenotype correlations, focusing on LLD, in a group of individuals with pure isolated hemihyperplasia/hemihypoplasia.

Results: We prospectively collected paired blood-tissue samples from 30 patients with pure isolated hemihyperplasia/hemihypoplasia who underwent surgery for LLD. Methylation-specific multiplex-ligation-dependent probe amplification assay (MS-MLPA) and bisulfite pyrosequencing for differentially methylated regions 1 and 2 (DMR1 and DMR2) on chromosome 11p15 were performed using the patient samples. Samples from patients showing no abnormalities in MS-MLPA or bisulfite pyrosequencing were analyzed by single nucleotide polymorphism (SNP) microarray and *CDKN1C* Sanger sequencing. We introduced a metric named as the methylation difference, defined as the difference in DNA methylation levels between DMR1 and DMR2. The correlation between the methylation difference and the predicted LLD at skeletal maturity, calculated using a multiplier method, was evaluated. Predicted LLD was standardized for stature. Ten patients (33%) showed epigenetic alterations in MS-MLPA and bisulfite pyrosequencing. Of these, six and four patients had epigenetic alterations related to BWS and SRS, respectively. The clinical diagnosis of hemihyperplasia/hemihypoplasia was not compatible with the epigenetic alterations in four of these ten patients. No patients showed abnormalities in SNP array or their *CDKN1C* sequences. The methylation difference was strongly correlated with the standardized predicted LLD in patients with epigenetic alterations ($r = 0.76$; $p = 0.01$).

Conclusions: Pure isolated hemihyperplasia and hemihypoplasia can occur as a spectrum of BWS and SRS. Although the accurate differentiation between isolated hemihyperplasia and isolated hemihypoplasia is important in tumor surveillance planning, it is often difficult to clinically differentiate these two diseases without epigenetic tests. Epigenetic tests may play a role in the prediction of leg length discrepancy, which would aid in treatment planning.

Background

Leg length discrepancy (LLD) is a common orthopedic condition that can cause various problems such as scoliosis and excessive stress on hip or knee joints [1, 2]. The etiology of LLD has long been of interest to orthopedic surgeons. Hemihyperplasia or hemihypoplasia, better known as congenital hemihypertrophy or hemihypotrophy to orthopedic surgeon, results in LLD by causing skeletal asymmetry [3-8]. Hemihyperplasia/hemihypoplasia can occur as a part of a recognized clinical syndrome or in isolation. Hemihyperplasia may be caused by neurofibromatosis type 1, Klippel-Trenaunay-Weber syndrome, Proteus syndrome, or Beckwith-Wiedemann syndrome (BWS), whereas hemihypoplasia may arise from Turner syndrome or Silver-Russell syndrome (SRS) [9, 10]. The etiologies of isolated hemihyperplasia/hemihypoplasia are not well-understood [1, 3, 9].

Epigenetics refers to modification of DNA, chromatin, and associated molecules that regulates gene expression without causing alterations to the DNA sequence itself. The major epigenetic mechanisms include DNA methylation, histone modifications, and RNA-mediated processes. Genomic imprinting is an epigenetic phenomenon resulting in monoallelic expression of a gene in a parent-of-origin-specific manner. Imprinted genes are typically arranged in clusters, where their expression is controlled by differentially methylated regions (DMR) between maternal and paternal chromosomes. BWS and SRS are opposite growth-affecting genomic imprinting disorders caused by opposite epigenetic alterations at the same chromosomal locus, to induce hemihyperplasia and hemihypoplasia, respectively (Table 1) [11, 12]. Epigenetic alterations in a cluster of imprinted genes on chromosome 11p15 are observed in approximately 80% of patients with BWS and 50% of patients with SRS [11, 12].

Normally, somatic growth is balanced between two imprinted genes on 11p15, *CDKN1C* and *IGF2* that negatively and positively regulates cell proliferation, respectively [13]. The former is transcribed from the maternal chromosome, whereas the latter is transcribed from the paternal chromosome (Fig. 1) [13]. BWS/SRS can occur by 1) gain of methylation (GOM) or loss of methylation (LOM) at DMR1 or DMR2 on 11p15, 2) 11p15 uniparental disomy (UPD) which refers to the inheritance or presence of two copies of a chromosome, or part of a chromosome, from one parent and no copies from the other parent, 3) germline mutations in *CDKN1C* or *IGF2*, or 4) 11p15 copy number variation [13-18]. GOM at DMR1, LOM at DMR2, paternal UPD 11p15, and *CDKN1C* loss-of-function mutations cause overgrowth through overexpression of *IGF2* and/or downregulation of *CDKN1C,* eventually resulting in BWS (Fig. 2) [13, 15, 18]. LOM at DMR1, maternal UPD 11p15, *CDKN1C* gain-of-function mutations, and *IGF2* loss-of-function mutations cause undergrowth through downregulation of *IGF2* and/or overexpression of *CDKN1C,* resulting in SRS (Fig. 3) [14, 16, 17].

Somatic mosaicism refers to the occurrence of two genetically distinct cells within an individual, derived from a postzygotic mutation. The phenotypes associated with mosaicism depend on the extent of the mosaic cell population. The international BWS consensus group introduced the concept of the Beckwith-Wiedemann spectrum (BWSp) in 2018 (Fig. 4) [11]. Because epigenetic alterations in BWS are frequently mosaic, variations in the expression of epigenetic alterations in different tissues can lead to the development of clinical features ranging from classic BWS to isolated hemihyperplasia. Similarly, the mosaic tissue distribution of 11p15 epigenetic alterations in SRS can produce various phenotypes, including isolated hemihypoplasia, as a part of Silver-Russell spectrum (SRSp) [8]. Considering the association between mosaicism and variations in phenotypes, we hypothesized that some of the nonsyndromic pure isolated hemihyperplasia/hypoplasia cases are caused by epigenetic changes on 11p15 and that the level of alteration in DNA methylation affects the extent of hemihyperplasia /hemihypoplasia, manifesting clinically as the degree of LLD.

In this study, we evaluated the underlying epigenetic alterations and potential epigenotype-phenotype correlations, focusing on LLD, in a group of individuals with pure isolated hemihyperplasia/hemihypoplasia.

Methods

Patients

Eighty-eight patients with hemihyperplasia/hemihypoplasia underwent epiphysiodesis or removal of hardware which had been inserted to correct LLD at a single tertiary-care pediatric center between December 2018 and March 2020 (Fig. 7). Because isolated hemihyperplasia/hemihypoplasia is a diagnosis of exclusion, 40 patients whose hemihyperplasia/hemihypoplasia may have been caused by other medical conditions were excluded following discussions between a pediatric orthopedic surgeon (C.H.S.) and clinical geneticist (J.M.K.). This group included 16 patients with genetic syndromes related to hemihyperplasia/hemihypoplasia, six patients with other congenital anomalies, five patients with skin pigmentation, four patients with peripheral nerve palsy, four patients with a history of fracture or tumor of the lower limbs, three patients with chromosomal abnormalities, and two patients with angular deformity of the lower limbs. Of the remaining 48 patients, 18 patients (38%) refused to participate. Twenty-three patients with hemihyperplasia and seven patients with hemihypoplasia constituted the study group. Hemihyperplasia and hemihypoplasia were classified by each surgeon preoperatively based on normative height, sitting height, and subischial leg length data [31, 32].

There were 17 male (57%) and 13 female (30%) patients. Twenty-eight patients underwent epiphysiodesis and two patients underwent tibial lengthening at an age of 11.6 ± 1.8 years (range 6.9–14.5 years). LLD was measured as the iliac crest height difference on a standing pelvic anteroposterior radiograph (Fig. 8). LLD at skeletal maturity was predicted using a multiplier method at the time of epiphysiodesis or tibial lengthening [33]. To standardize the predicted LLD for stature, the predicted LLD was divided by the height at the time of epiphysiodesis or tibial lengthening.

Sample Collection

Because of somatic mosaicism, 11p15 epigenetic alterations in individual patients may differ between cells from different tissues, and acquisition of tissue samples from overgrown/undergrown regions increases the likelihood of finding epigenetic alterations [7]. Therefore, at the time of operation, we collected paired blood-tissue samples composed of 5 mL of peripheral blood and a small amount of dermis, fat, and muscle. Tissue samples were obtained through the incision made for epiphysiodesis or hardware removal. Muscles were obtained from the vastus lateralis using a 3-mm punch in 17 patients undergoing operations at the distal femur and proximal tibia and in 11 patients at the distal femur, and from the tibialis anterior in two patients undergoing operations at the tibia. The mean age of the patients was 12.9 ± 1.8 years (range 7.9–15.7 years) at the time of sample acquisition.

Molecular Testing

Molecular testing to identify the genetic and epigenetic alterations responsible for BWS/SRS, 1) GOM or LOM at DMR1 or DMR2 on 11p15, 2) UPD 11p15, 3) germline mutations in *CDKN1C*, or 4) 11p15 copy number variation [13–18], was performed. MS-MLPA assay and bisulfite pyrosequencing for DMR1 and DMR2 on 11p15 were performed for all patients (Fig. 9). MS-MLPA can detect methylation alteration, UPD, and copy number variation, and bisulfite pyrosequencing can detect methylation alteration and UPD. Samples from patients who did not show abnormalities of any tissue in MS-MLPA and bisulfite pyrosequencing were further analyzed by SNP microarray which can detect UPD and copy number variation and *CDKN1C* Sanger sequencing. The details of molecular testing are shown in Additional file 2.

Statistical Analysis

The correlation between the methylation difference and standardized predicted LLD was evaluated using Spearman correlation test. Significance was set at $p < 0.05$. Statistical analysis was performed using STATA 15.1 (Stata Corp, College Station, TX, USA).

Results

Methylation-specific multiplex-ligation-dependent probe amplification (MS-MLPA) assay detected epigenetic alterations in 9/30 patients (30%), and bisulfite pyrosequencing detected in 10/30 patients (33%; Table 2). All patients who had epigenetic alterations identified by MS-MLPA also had the same alterations identified by bisulfite pyrosequencing. Epigenotypes identified in bisulfite pyrosequencing were consistent across the samples tested in each patient (Additional file 1). No patients showed abnormal single nucleotide polymorphism (SNP) array or *CDKN1C* Sanger sequencing results. Of the 10 patients with epigenetic alterations, seven had alterations in both the blood and tissue samples, two had alterations only in the tissue samples, and one had alterations only in the blood sample.

Of the 10 patients with epigenetic alterations, eight had GOM or LOM at DMR1 or DMR2 on 11p15 and two had a UPD 11p15 pattern. No patients had germline mutations in *CDKN1C* or 11p15 copy number variation. Of these 10 patients, six had epigenetic alterations of BWS and four had alterations of SRS (Table 2) (Fig. 5). The epigenotypes of six patients with BWSp were LOM at DMR2 in four patients, GOM at DMR1 in one, and paternal UPD 11p15 pattern (both GOM at DMR1 and LOM at DMR2) in one. The epigenotypes of four patients with SRSp were LOM at DMR1 in three patients and maternal UPD 11p15 pattern (both LOM at DMR1 and GOM at DMR2) in one. The clinical diagnosis of six patients with BWSp was hemihyperplasia in four and hemihypoplasia in two. The clinical diagnosis of four patients with SRSp was hemihyperplasia in two and hemihypoplasia in two.

We introduced a metric named as the methylation difference, defined as the difference in DNA methylation levels between DMR1 and DMR2 in the same tissue using bisulfite pyrosequencing. Because GOM at DMR1 promotes growth via transcription of *IGF2* and GOM at DMR2 restricts growth via transcription of *CDKN1C* [13], the methylation difference would determine the direction of growth. We assumed that the methylation difference is correlated with the severity of predicted LLD at skeletal maturity. Among the blood, dermis, fat, and muscle samples, fat samples were used to calculate

the methylation difference because we hypothesized that local tissue that had developed from mesoderm would contribute more to leg length than peripheral blood or local tissue from the ectoderm, and because obtaining a larger amount of fat causes less morbidity than obtaining muscle.

The methylation difference showed a moderate correlation with the standardized predicted LLD at skeletal maturity in all patients (r = 0.55; p = 0.002) [19]. The mean standardized predicted LLD (± standard deviation [SD]) was 2.5 ± 1.5% (range 1.1–8.7%), and the methylation difference was 2.2 ± 2.2 SD (range 0–10.3 SD). The methylation difference was strongly correlated with the standardized predicted LLD in 10 patients with methylation alterations (r = 0.76; p = 0.01) (Fig. 6).

Discussion

We demonstrated that pure isolated hemihyperplasia and hemihypoplasia can occur as BWSp and SRSp, and the methylation difference is valuable for predicting the severity of future LLD. This is the first study to identify an association between the severity of LLD and epigenetic alterations, providing a basis for understanding the development of idiopathic LLD and value of epigenetic tests for patients with isolated hemihyperplasia/hemihypoplasia.

Several previous studies have shown that patients with isolated hemihyperplasia/hemihypoplasia may have epigenetic alterations that are found in BWS/SRS [6, 7, 20–22]. Shuman et al. reported eight patients with paternal UPD 11p15 and three with LOM at DMR2 among 51 patients with isolated hemihyperplasia [7]. Bliek et al. reported a series of eight patients with clinical features ranging from isolated hemihypoplasia to full-spectrum SRS who had LOM at DMR1 [20]. However, all previous studies of “isolated hemihyperplasia/hemihypoplasia” included both patients with pure isolated hemihyperplasia/hemihypoplasia and patients with skin pigmentation or other BWS/SRS clinical features who did not fit the definition of “isolated” [6, 7, 20–22]. Previous studies did not obtain tissue samples from every patient, which may have resulted in false negative results, considering the mosaic distribution of the affected cells. To the best of our knowledge, this is the first study to reveal epigenetic alterations on 11p15 using paired blood-tissue samples in a group of individuals with “pure” isolated hemihyperplasia/hemihypoplasia.

Although *CDKN1C* mutations have been identified in 8% of patients with BWS [13], we did not observe these mutations. This may be because the underlying molecular defect of *CDKN1C* mutations is a germline mutation that affects all cells in the body rather than a somatic mosaicism and because our study population had isolated hemihyperplasia/hemihypoplasia. Previous studies of BWS/SRS reported the extreme rarity of hemihyperplasia/hemihypoplasia in patients with *CDKN1C* mutations [15, 23]. *CDKN1C* sequencing is therefore thought to be unnecessary in patients with pure isolated hemihyperplasia/hemihypoplasia.

Because the epigenetic alteration exists in a mosaic form in BWSp/SRSp, it is possible that the proportion of cells with altered methylation is high in hyperplastic/hypoplastic tissues. Therefore, the level of DNA methylation alteration may differ even in patients with the same epigenotype, leading to differences in the phenotype severity. We quantitatively represented this “methylation burden” as the methylation difference between DMR1 and DMR2, which was shown to correlate with the severity of LLD. Particularly, UPD affects methylation at both DMR1 and DMR2 in opposite directions, resulting in a large methylation difference. In this study, two patients showed a UPD pattern. One patient with a maternal UPD pattern had 57 mm of predicted LLD, and another patient with paternal UPD had 112 mm of predicted LLD. A previous case report described a patient with paternal UPD 11p15 who had hemihyperplasia affecting the right leg and showed 50 mm of LLD at an early age of 8.3 years [24]. Therefore, UPD appears to be associated with severe phenotypes. This is the first study to quantitatively analyze the association between the degree of epigenetic alteration and severity of phenotype in patients with BWSp/SRSp. This strategy may be applicable in patients with BWSp to assess tumor development risk.

It may be controversial whether and when to perform epigenetic tests on patients with pure isolated hemihyperplasia/hemihypoplasia. If we identify methylation alterations via epigenetic tests, we can reassure patients that the risk of recurrence of hemihyperplasia/hemihypoplasia in their offspring is negligible [18]. If a UPD pattern is observed,

we can inform patients of the possibility of lengthening procedures, rather than an operative procedure that suppresses longitudinal bone growth, or epiphysiodesis, for large LLD. In this study, we confirmed epigenetic alterations using peripheral blood samples in 8/10 patients with methylation defect. Therefore, epigenetic testing using peripheral blood can be performed at an early age and using blood and tissue samples at operation for LLD.

Because there are no clinical features other than a size difference between the sides in pure isolated hemihyperplasia/hemihypoplasia, it is difficult to differentiate these two diseases clinically. Unlike in hemihyperplasia, LLD in hemihypoplasia is rarely more than 25 mm and therefore does not require surgery [3]. However, in this study, the predicted LLDs of two of four patients with the epigenotype of SRS were 57 and 32 mm. Therefore, LLD due to hemihypoplasia in SRSp can exceed 25 mm, and we should not differentiate these two diseases based on the severity of LLD.

In this study, the clinical diagnosis of hemihyperplasia/hemihypoplasia was not compatible with epigenetic alterations in four of 10 patients. Other studies have also found LOM at DMR1, which should cause hemihypoplasia in patients diagnosed with isolated hemihyperplasia [5, 8]. Because screening for embryonal tumors is recommended only for patients with hemihyperplasia, and not for patients with hemihypoplasia [11, 12], the differentiation of isolated hemihyperplasia from hemihypoplasia has serious prognostic implications. However, without epigenetic tests, this discrimination is nearly impossible, except in a few patients with extreme phenotypes. This problem highlights the need to perform epigenetic tests in patients with isolated hemihyperplasia/hemihypoplasia.

LLD is a common orthopedic condition, which frequently occurs for unknown causes [25]. This study provides a basis for understanding the molecular mechanism underlying idiopathic LLD. When epigenetic alterations of BWS/SRS are confined to the thigh and/or leg due to somatic mosaicism, the condition appears as idiopathic LLD. A considerable portion of idiopathic LLD appears to occur as a BWSp/SRSp.

This study has several limitations. First, we did not examine other genetic mechanisms affecting growth [26–28]. These mechanisms can be confounding factors that may block the correlation between the methylation difference and predicted LLD. In the first five consecutive patients, we examined genes involved in the PI3K/AKT/mTOR pathway, which is also known to cause syndromic hemihyperplasia [27], using tissue-blood paired high-depth exome sequencing. However, we found no meaningful tissue-specific variant in any sample and thus discontinued the examination. Second, tissue samples could be obtained only from the operated legs for ethical reasons. Because the differentiation between hemihyperplasia and hemihypoplasia is confusing, the leg operated on may not be the affected leg. However, we examined peripheral blood and multiple tissues to confirm our results. Third, the predicted LLD calculated by the multiplier method may differ from the true LLD [29], although this is one of the most frequently used methods for predicting LLD [30].

Conclusions

Pure isolated hemihyperplasia and hemihypoplasia can occur as a spectrum of BWS and SRS. Although the accurate differentiation between isolated hemihyperplasia and isolated hemihypoplasia is important in tumor surveillance planning, it is often difficult to clinically differentiate these two diseases without epigenetic tests. Epigenetic tests may play a role in the prediction of leg length discrepancy, which would aid in treatment planning.

Abbreviations

BWS: Beckwith-Wiedemann syndrome; BWSp: Beckwith-Wiedemann spectrum; DMR1: Differentially methylated region 1; DMR2: Differentially methylated region 2; GOM: Gain of methylation; LLD: Leg length discrepancy; LOM: Loss of methylation; MS-MLPA: Methylation-specific multiplex-ligation-dependent probe amplification assay; SD: standard

deviation; SNP: Single nucleotide polymorphism; SRS: Silver-Russell syndrome; SRSp: Silver-Russell spectrum; UPD: uniparental disomy.

Declarations

Ethics approval and consent to participate

This study was carried out under an ethics approval from Seoul National University College of Medicine/Seoul National University Hospital Institutional Review Board (IRB Number: H-1811-030-983). All participants in this study provided informed written consents for participation in molecular testing from their donated blood and tissue samples, after viewing documentation about the studies.

Consent for publication

We obtained written informed consent from the patients and the patients' parents to publish patients' clinical and molecular information.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CHS, IHC, and JMK conceived and planned the study. CHS, CML, WJY, and TJC created the cohort and provided patient samples. JMK performed the molecular testing (MS-MLPA, sequencing, and SNP microarray). HWK and CHS collected and analyzed the data. CHS and JMK drafted the manuscript and revised it in communication with CML, HWK, WJY, TJC, and IHC. All authors read and approved the final manuscript.

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Tables

Table 1. Typical clinical features of Beckwith-Wiedemann syndrome and Silver-Russell syndrome

Beckwith-Wiedemann syndrome	Silver-Russell syndrome
Hemihyperplasia	Hemihypoplasia
Large for gestational age	Small for gestational age
Polyhydramnios and/or placentomegaly	Oligohydramnios
Macroglossia	Postnatal growth retardation
Exomphalos or umbilical hernia	Relative macrocephaly at birth
Hyperinsulinism*/transient hypoglycemia†	Protruding forehead at the age of 1–3 years
Facial nevus flammeus	Triangular face
Ear creases and/or pits	
Wilms tumor, hepatoblastoma, nephroblastoma, neuroblastoma, adrenocortical carcinoma, or pheochromocytoma	

*Lasting >1 week and requiring escalated treatment. †Lasting < 1week.

Table 2. Clinical features and results of molecular tests of patients (n = 30)

Patient No.	Sex	Age* (*yr*)	Predicted LLD† (*mm*)	Standardized predicted LLD‡ (%)	Epigenotype	MS-MLPA for 11p15	Bisulfite pyrosequencing		
							DMR1§ (*SD*)	DMR2§ (*SD*)	Methylation difference‖(*SD*)
Hemihyperplasia									
1	F	10.8	25	1.8	N	N	-0.72 (B)	0.74 (B)	0
2	F	11	33	2.2	N	N	0.56 (F)	1.82 (F)	2.38
3	M	12.8	51	3.1	DMR1-GOM	DMR1-GOM	2.63 (F)	-0.68 (F)	3.31
4	M	14.5	26	1.5	N	N	-1.01 (F)	1.43 (F)	2.44
5	M	13	20	1.1	N	N	-0.77 (B)	-1.67 (B)	0.02
6	M	6.9	112	8.7	pUPD 11p15	DMR1-GOM & DMR2-LOM	3.55 (F)	-6.72 (F)	10.27
7	M	14	20	1.2	DMR2-LOM	DMR2-LOM	0.39 (B)	-5.59 (B)	3.1
8	M	8.4	43	3.2	N	N	1.53 (S)	-1.52 (S)	0.71
9	M	14.4	40	2.5	N	N	0.08 (F)	-1.51 (F)	1.59
10	M	11.2	30	2	N	N	-0.33 (S)	-0.78 (S)	0.41
11	M	12.4	34	2.3	N	N	-1.39 (S)	-0.04 (S)	1.33
12	M	10.8	40	2.8	N	N	0.94 (S)	-0.81 (S)	0.86
13	M	11	22	1.5	N	N	0.31 (M)	-0.74 (M)	0.36
14	M	14.4	24	1.3	DMR1-LOM	DMR1-LOM	-2.12 (B)	-0.9 (B)	0.4
15	F	11.1	32	2.2	DMR1-LOM	DMR1-LOM	-2.24 (F)	1.29 (F)	3.53
16	M	12.1	38	2.5	N	N	-1.46 (F)	1.69 (F)	3.15
17	M	13.6	20	1.1	N	N	-0.72 (B)	1.54 (B)	0.97
18	F	12	26	1.6	N	N	-1.93 (B)	1.91 (B)	2.15
19	F	12.1	25	1.6	N	N	-0.68 (B)	1.58 (B)	1.98

20	F	10.6	35	2.4	N	N	1.32 (M)	-1.62 (M)	0.95
21	F	12	25	1.6	N	N	-0.89 (B)	0.89 (B)	0.09
22	F	11.5	26	1.7	N	N	-1.12 (S)	1.17 (S)	0.59
23	F	7.9	61	4.8	DMR2-LOM	DMR2-LOM	-1.65 (B)	-14.72 (B)	7.77
Hemihypoplasia									
1	M	12	43	2.9	N	N	0.97 (M)	-0.91 (M)	0.99
2	F	10.1	57	4.1	mUPD 11p15	N	-2.65 (S)	2.71 (S)	3.27
3	M	11.7	44	3	DMR2-LOM	DMR2-LOM	-0.02 (B)	-3.25 (B)	3.08
4	M	12.2	26	1.7	N	N	0.94 (M)	-1.47 (M)	0.67
5	F	11.4	54	3.5	DMR2-LOM	DMR2-LOM	0.69 (F)	-2.51 (F)	3.2
6	F	11.2	20	1.4	DMR1-LOM	DMR1-LOM	-3.96 (B)	0.18 (B)	2.31
7	M	12.3	37	2.5	N	N	-1.65 (S)	1.6 (S)	3.08

*Age at epiphysiodesis or tibial lengthening was presented. †Leg length discrepancy at skeletal maturity was predicted using the multiplier method. ‡Predicted leg length discrepancy was divided by the height at the time of epiphysiodesis or tibial lengthening. §Among blood and tissue samples, results of the sample with the maximum methylation difference were described. ||Methylation difference was calculated in fat samples as follows: |altered DNA methylation level (SD) at DMR1 – DMR2|.

LLD = leg length discrepancy, MS-MLPA = methylation-specific multiplex-ligation-dependent probe amplification, DMR1 = differentially methylated region 1, DMR2 = differentially methylated region 2, SD = standard deviation, N = normal, GOM = gain of methylation, LOM = loss of methylation, pUPD = paternal uniparental disomy, mUPD = maternal uniparental disomy, B = blood, F = fat, S = skin, and M = muscle.

Figures

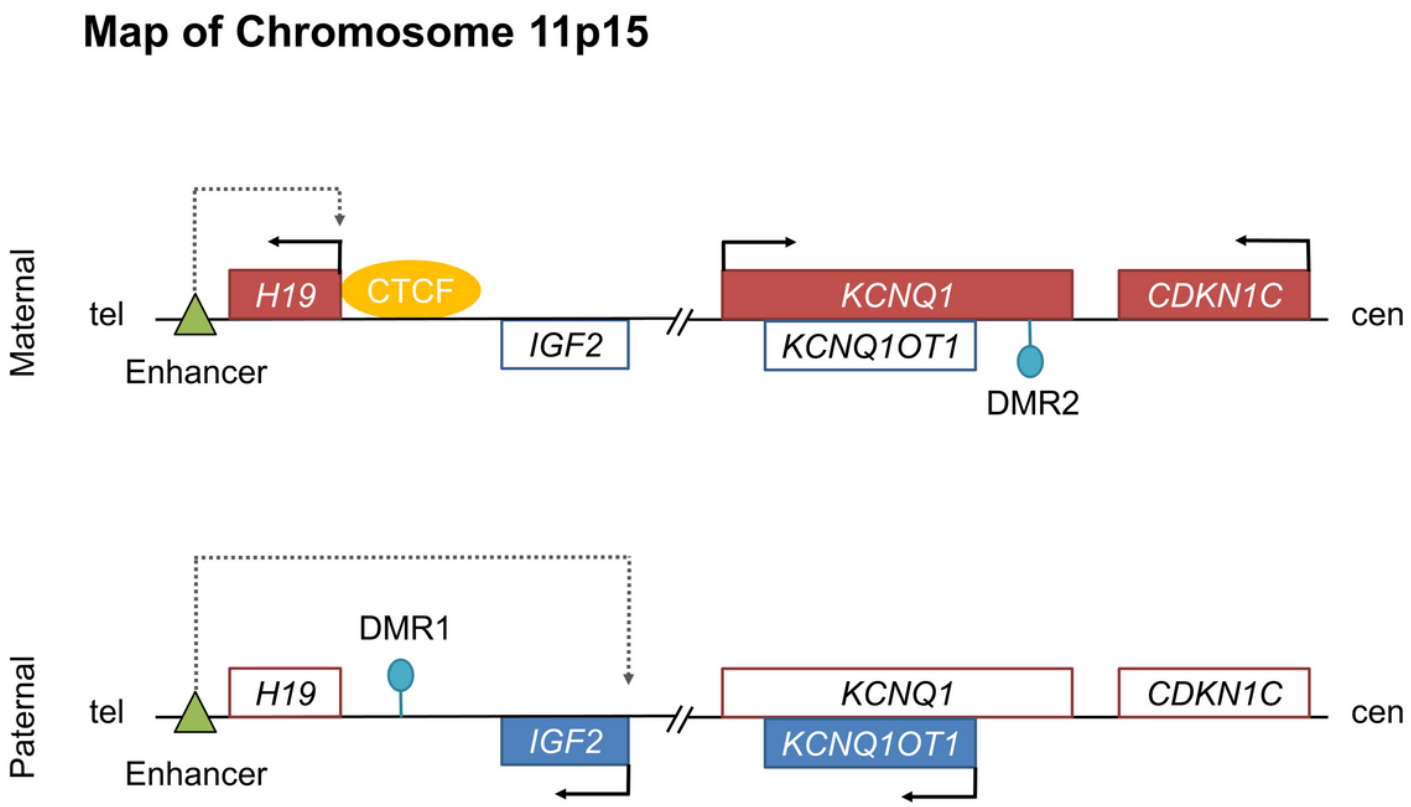

Figure 1

A map of chromosome 11p15. Normally, the maternal allele is methylated at differentially methylated region 2 (DMR2), and the paternal allele is methylated at differentially methylated region 1 (DMR1). Unmethylated DMR1 on the maternal chromosome permits binding of the insulator protein CTCF, which blocks access of enhancers to the IGF2 promoter. Therefore, the maternal copy of H19 uses enhancers and is transcribed. Methylated DMR1 on the paternal chromosome prevents binding of the CTCF. Therefore, enhancers can access the IGF2 promoter, which is transcribed. On the maternal chromosome, DMR2 is methylated, and KCNQ1 and CDKN1C are transcribed. On the paternal chromosome, DMR2 is unmethylated, and KCNQ1OT1 is transcribed. IGF2 positively and CDKN1C negatively regulates cell growth and proliferation. Green triangles indicate enhancers. Lollipops indicate methylated DMR. Genes normally expressed from the maternal chromosome are depicted as red boxes, and genes normally expressed from the paternal chromosome are depicted as blue boxes. Arrows indicate the orientation of transcription. Tel = telomere. Cen = centromere.

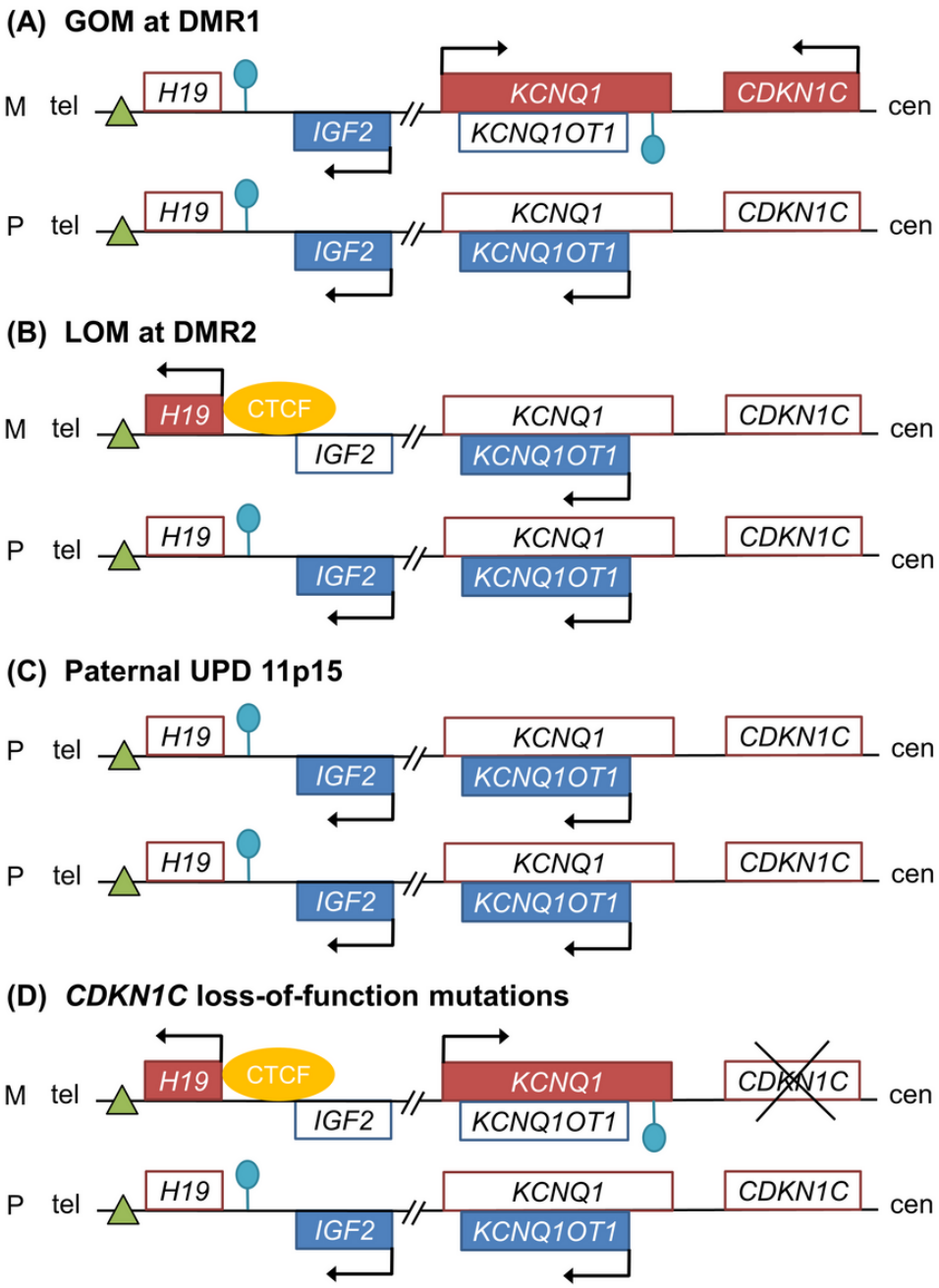

Figure 2

Molecular mechanism of Beckwith-Wiedemann syndrome. a Gain of methylation (GOM) at differentially methylated region 1 (DMR1) on the maternal chromosome results in downregulation of H19 and expression of IGF2. b Loss of methylation (LOM) at differentially methylated region 2 (DMR2) on the maternal chromosome results in downregulation of KCNQ1 and CDKN1C and expression of KCNQ1OT1. c Paternal uniparental disomy (UPD) occurs when a patient has two copies of the paternal chromosome and none of the maternal chromosome. Paternal UPD 11p15 results in overexpression of IGF2 in addition to downregulation of KCNQ1 and CDKN1C. d CDKN1C loss-of-function mutations on the maternal chromosome also result in Beckwith-Wiedemann syndrome. Green triangles indicate enhancers. Lollipops indicate methylated imprinting centers. Genes normally expressed from the maternal chromosome are depicted as red boxes, and genes normally expressed from the paternal chromosome are depicted as blue boxes. Arrows indicate the orientation of transcription. M = maternal. P = paternal. Tel = telomere. Cen = centromere.

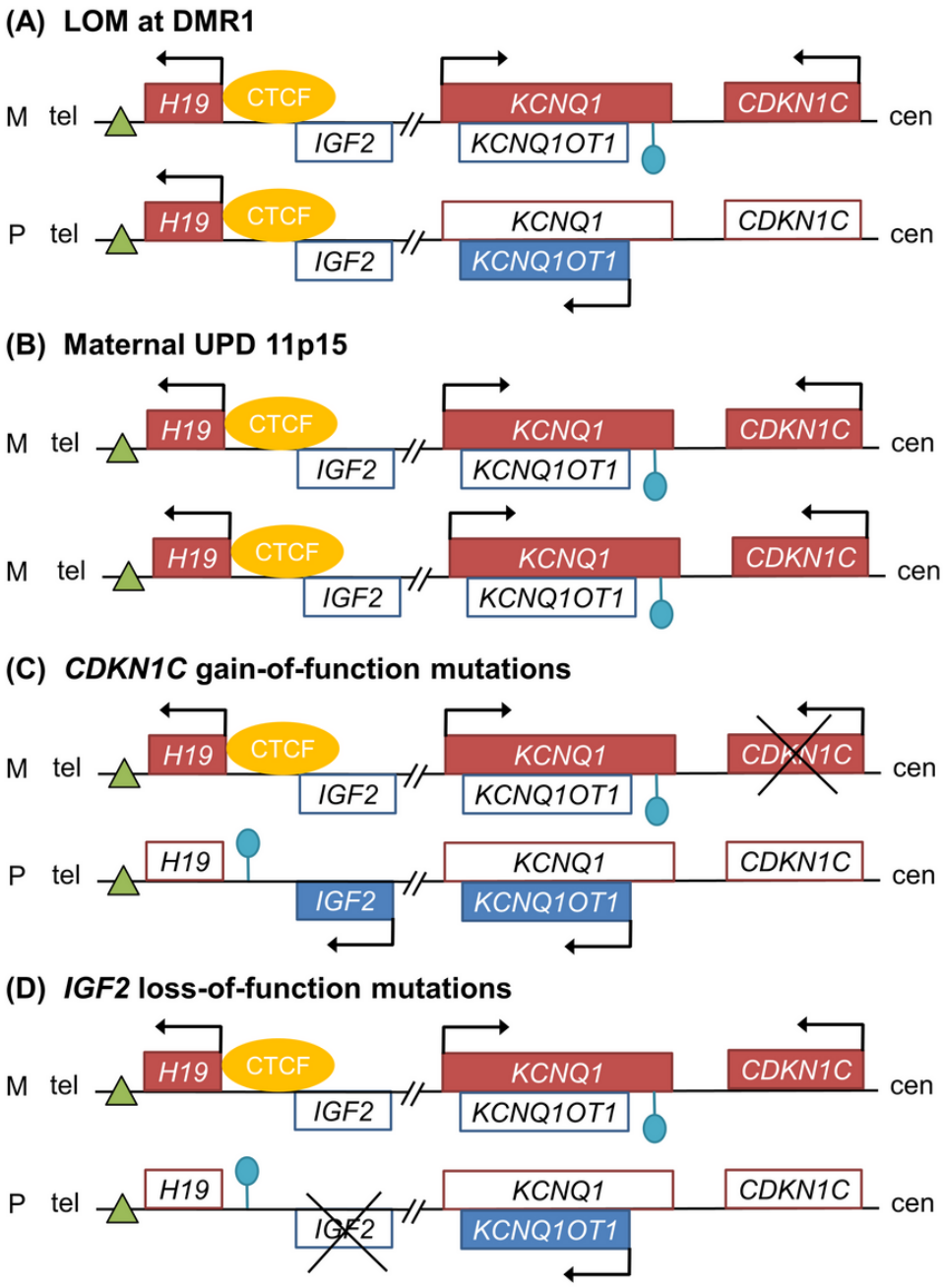

Figure 3

Molecular mechanism of Silver-Russell syndrome. a Loss of methylation (LOM) at differentially methylated region 1 (DMR1) on the paternal chromosome results in expression of H19 and downregulation of IGF2. b Maternal uniparental disomy (UPD) occurs when a patient has two copies of the maternal chromosome and none of the paternal chromosome. Maternal UPD 11p15 results in downregulation of IGF2 in addition to overexpression of KCNQ1 and CDKN1C. c CDKN1C gain-of-function mutations on the maternal chromosome and d IGF2 loss-of-function mutations on the paternal chromosome also result in Silver-Russell syndrome. Green triangles indicate enhancers. Lollipops indicate methylated imprinting centers. Genes normally expressed from the maternal chromosome are depicted as red boxes, and genes normally expressed from the paternal chromosome are depicted as blue boxes. Arrows indicate the orientation of transcription. M = maternal. P = paternal. Tel = telomere. Cen = centromere.

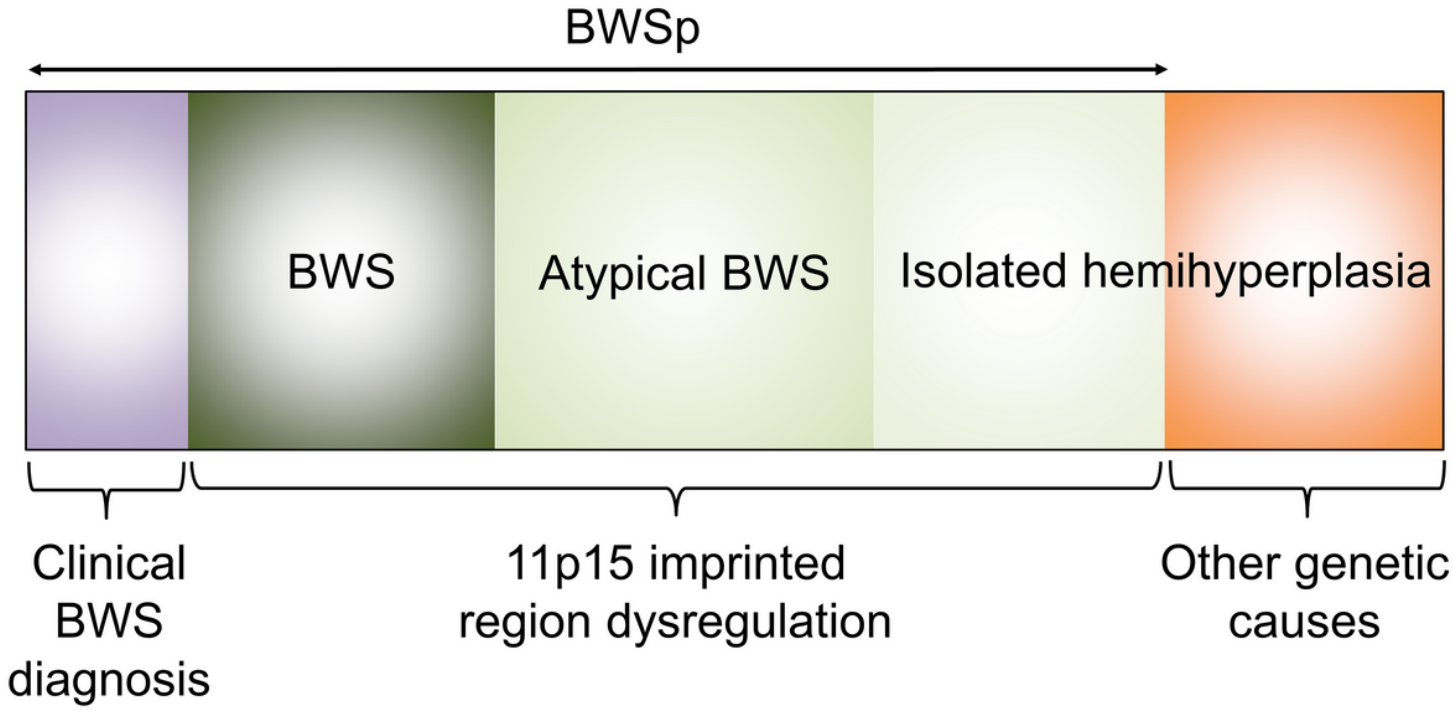

Figure 4

Beckwith-Wiedemann spectrum. The Beckwith-Wiedemann spectrum (BWSp) includes patients who meet the clinical diagnostic criteria of Beckwith-Wiedemann syndrome (BWS) with or without (epi)genetic alterations at the BWS locus on chromosome 11p15, patients with fewer features of BWS and (epi)genetic alterations at 11p15, and patients with isolated hemihyperplasia and (epi)genetic alterations at 11p15.

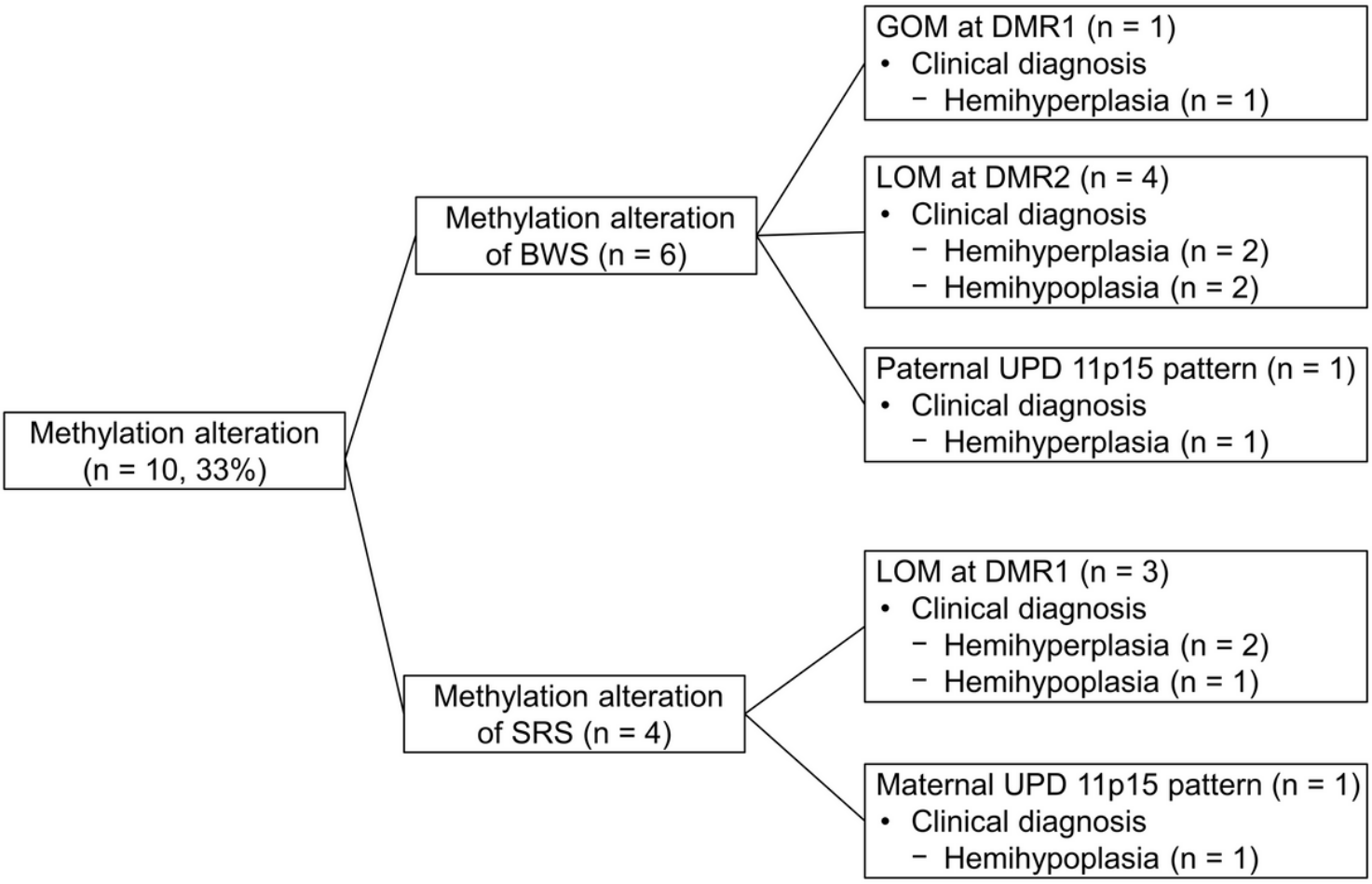

Figure 5

Clinical diagnosis and results of molecular tests of patients.

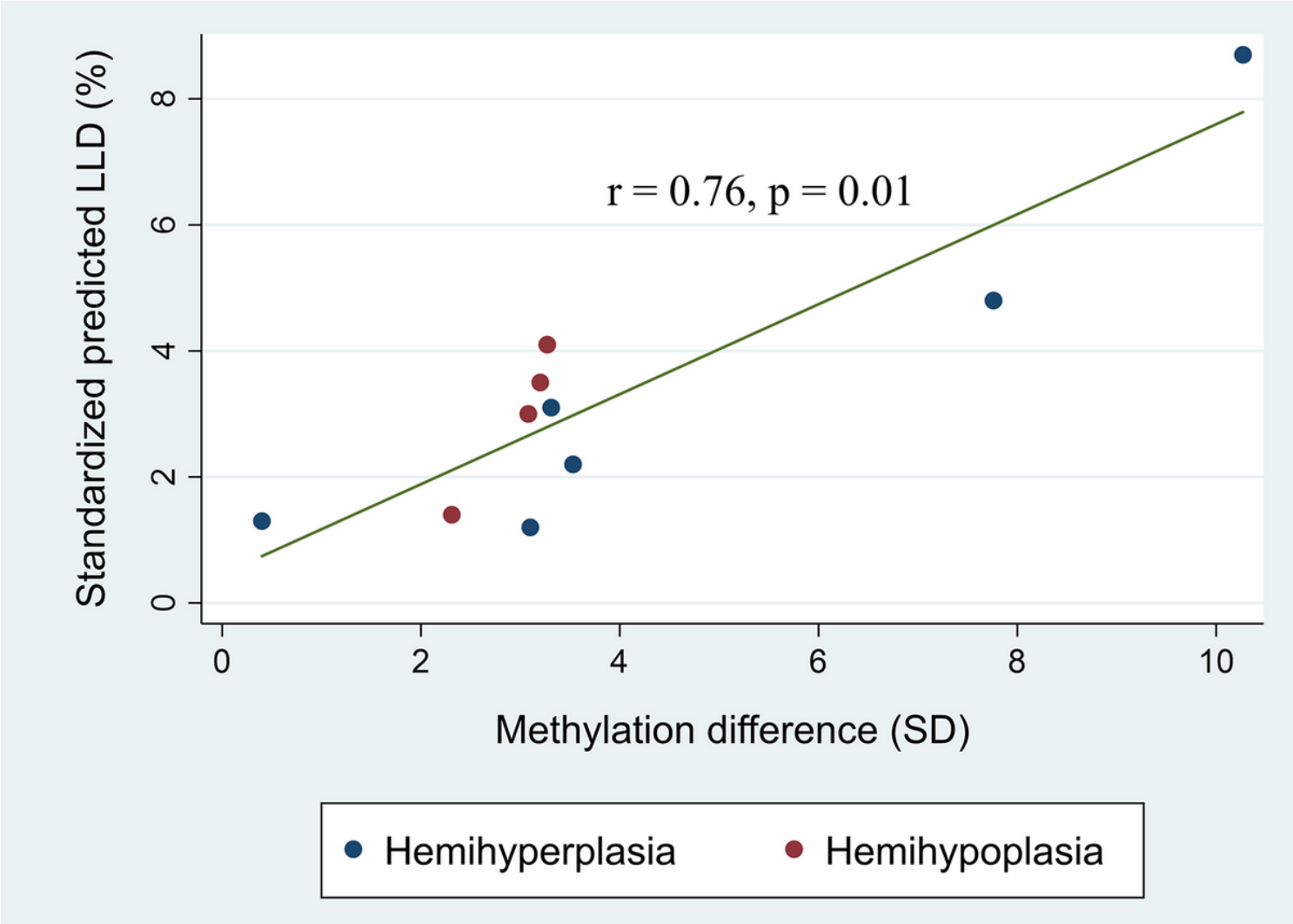

Figure 6

Scatterplot of the methylation difference and predicted leg length discrepancy (LLD) at skeletal maturity in patients with methylation alterations.

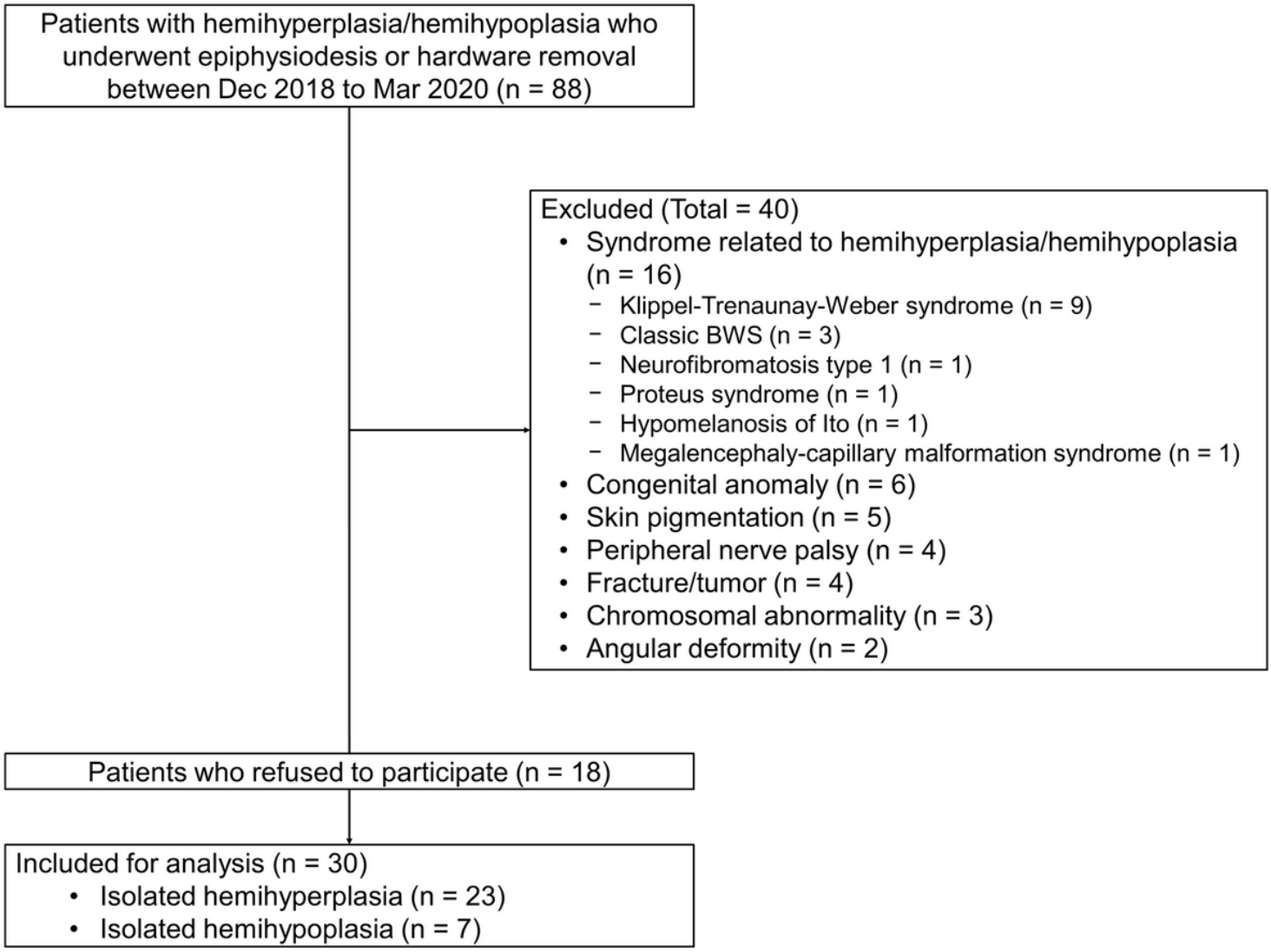

Figure 7

Flowchart of the study population.

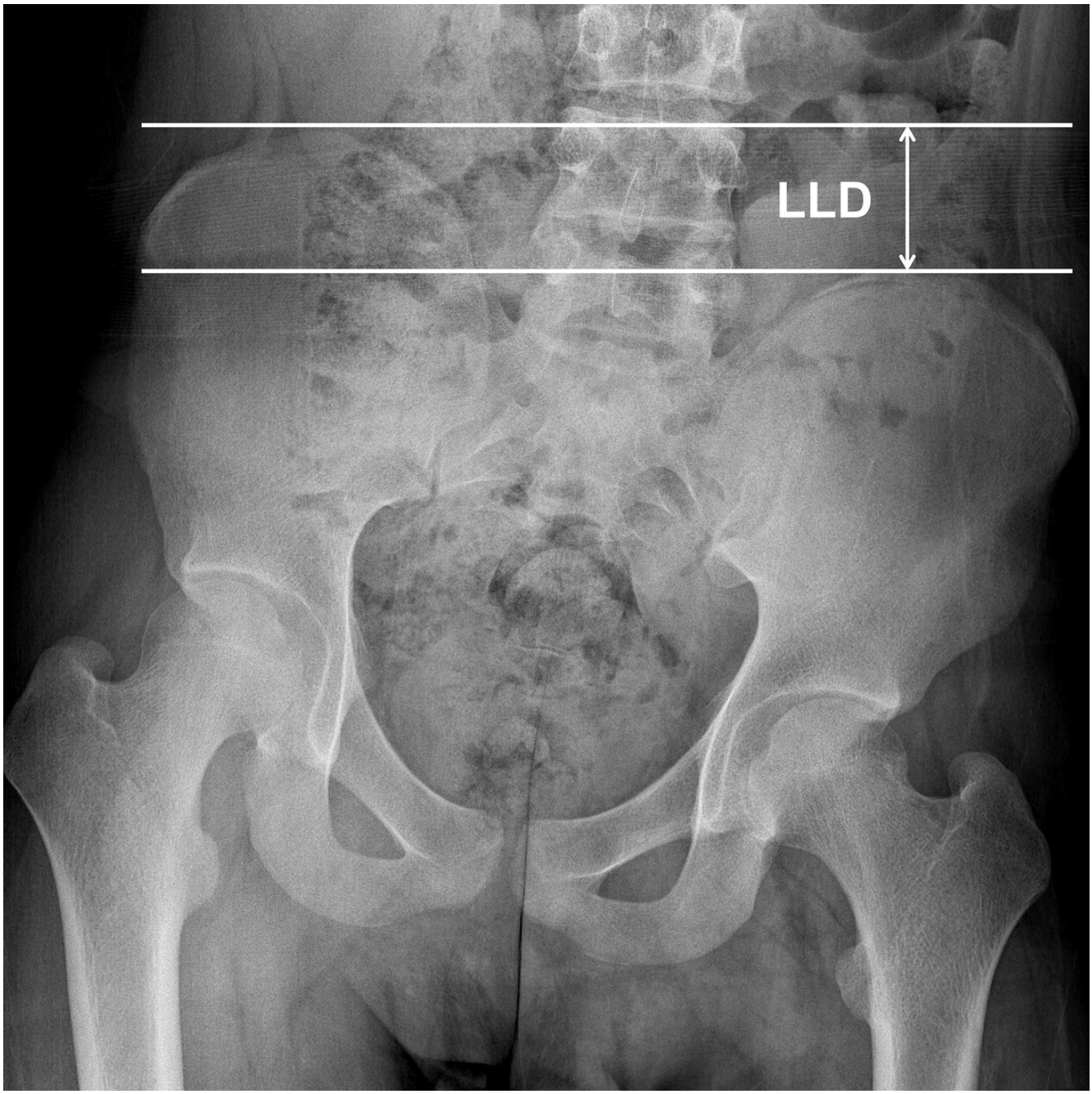

Figure 8

Measurement of leg length discrepancy (LLD) on a standing pelvic anteroposterior radiograph.

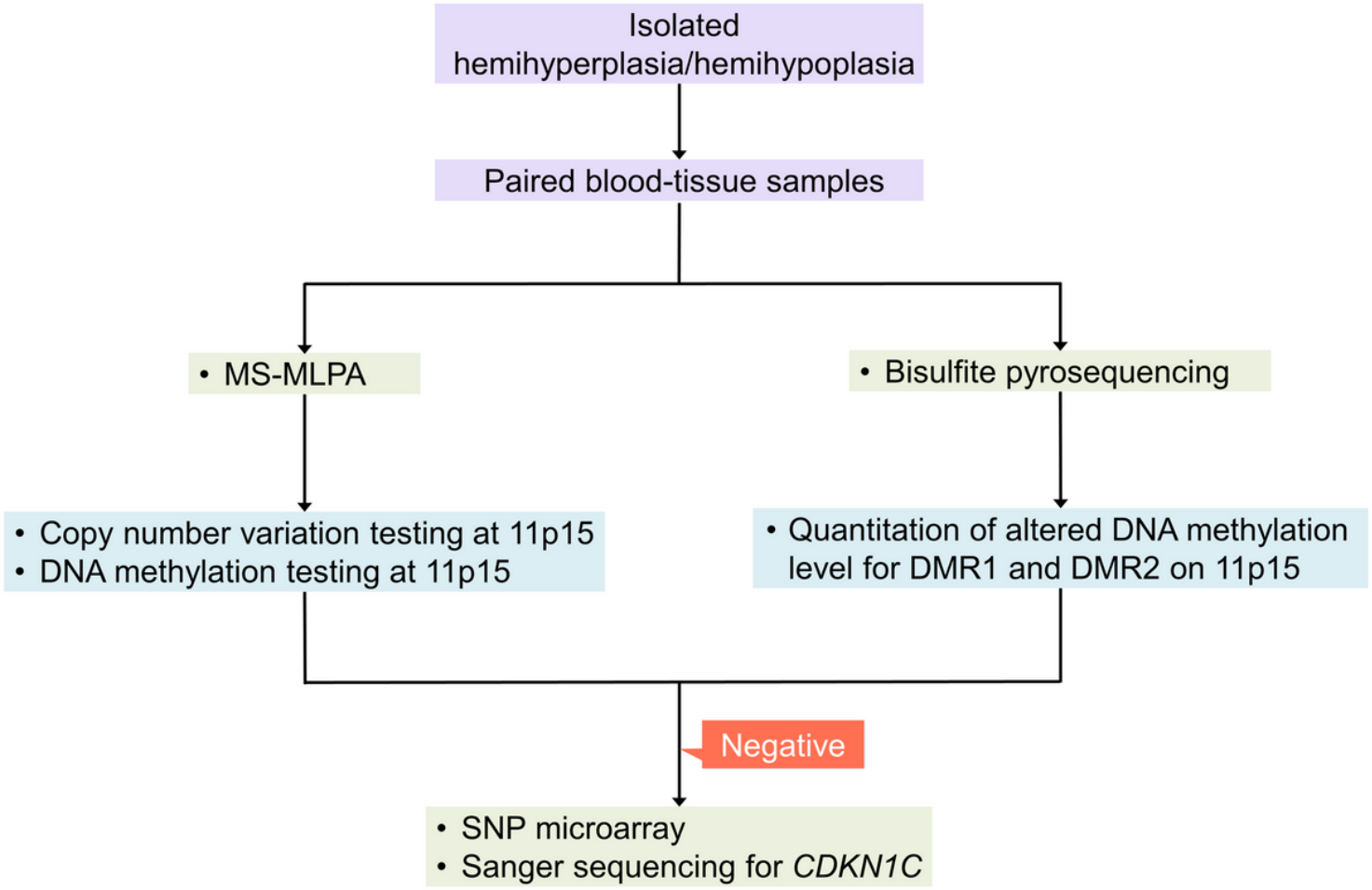

Figure 9

Flowchart of the molecular test.

Supplementary Files

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- Additionalfile1.docx
- Additionalfile2.docx