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Clinical phenotypes, bone-age delay, and exploratory coding-variant architecture in idiopathic short stature: a single-center integrative analysis

Lijun Hao
Xi'an People's Hospital (Xi'an Fourth Hospital)
Yifan Yang
The Second Affiliated Hospital of Xi'an Jiaotong University
Biyao Lian
The Second Affiliated Hospital of Xi'an Jiaotong University
Wenxin Feng
Shangluo Central Hospital
Chunyan Yin
The Second Affiliated Hospital of Xi'an Jiaotong University
Yuesheng Liu
lothardan@163.com

The Second Affiliated Hospital of Xi'an Jiaotong University

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Abstract

Background.

Idiopathic short stature (ISS) is a diagnosis of exclusion applied to children with height more than two standard deviations below the population mean in whom no causative disorder is identified. ISS is clinically and genetically heterogeneous, and integrating routinely collected clinical data with sequencing data may help to generate testable hypotheses about its architecture. We performed a single-center, exploratory analysis combining structured clinical variables, phenotypes extracted from free-text notes, and sample-level coding-variant matrices in children with ISS and normal-height clinic controls.

Methods.

Children evaluated at a single pediatric clinic were classified as ISS (height SDS < − 2; n = 25) or normal height (height SDS > − 1; n = 22); children with intermediate height SDS were excluded. Sequencing matrices were available for 22 ISS and 7 normal-height children. Clinical variables (IGF-1, IGFBP-3, bone-age difference [BAD]), free-text–derived phenotype categories, mutation burden, ISS-enriched genes (nominal Fisher's exact P < 0.05), phenotype subgroups, and a seed-gene protein–protein interaction network were analyzed with Wilcoxon, Fisher's exact, and Spearman tests, followed by exploratory Gene Ontology over-representation analysis.

Results.

ISS children had lower IGF-1 (median 101.4ng/ml vs 351.2ng/ml), lower IGFBP-3 (3.19ug/ml vs 5.20ug/ml), and more delayed bone age (BAD − 2.1 vs 0.1 years) than normal-height children. Facial, skeletal/limb, and neurodevelopmental phenotype categories extracted from clinical notes were enriched in ISS. Within ISS, height SDS correlated with BAD ($r_s = 0.51$), and familial short stature was associated with greater bone-age delay. Total and high-impact coding-variant burdens were lower in ISS than in the small normal-height sequenced set. Thirty-five unique genes were nominally enriched for variants in ISS; exploratory Gene Ontology terms included chromatin-remodeling complexes involving EP400 and BPTF. A seed-gene STRING network placed SRCAP adjacent to EP400 and BPTF; SRCAP missense variants were absent in sequenced ISS (0/22) but present in 4/7 sequenced normal-height children, a contrast that cannot be interpreted as pathogenic or protective because variant matrices were not filtered for rarity or predicted pathogenicity.

Conclusions.

This hypothesis-generating, single-center study links clinical severity of ISS to bone-age delay and nominates chromatin-remodeling genes as candidates for follow-up in larger, stringently filtered cohorts.

Findings require independent replication and should not be used for clinical decision-making.

1. Introduction

Short stature is one of the most common reasons for referral to pediatric endocrine clinics. When a child's height lies more than two standard deviations (SD) below the mean for age, sex, and population, and systematic evaluation fails to identify an endocrine, systemic, nutritional, chromosomal, or skeletal cause, the descriptive label idiopathic short stature (ISS) is applied 1 2. The 2008 international consensus statement and subsequent guideline updates define ISS by this height threshold and explicitly frame it as a diagnosis of exclusion rather than a discrete disease entity 1 3. Because ISS is operationalized by an auxological cutoff, affected children form a clinically heterogeneous group in which familial short stature, constitutional delay of growth and puberty, and unrecognized monogenic or syndromic conditions are intermixed 2 3.

Over the past decade, genomic studies have progressively eroded the notion that ISS is genetically inert. Targeted sequencing, copy-number analysis, and exome sequencing in children labeled as ISS or as nonsyndromic short stature have implicated genes acting in the growth hormone (GH)–insulin-like growth factor 1 (IGF-1) axis, the growth-plate extracellular matrix and paracrine signaling systems, and fundamental cellular processes such as chromatin regulation 4 5 6. Diagnostic yields vary with ascertainment, but studies of selected short-stature cohorts report monogenic explanations in a substantial minority of patients, particularly when additional features such as mild skeletal disproportion, dysmorphism, or neurodevelopmental findings are present 7 8. Recent reviews of human growth genetics emphasize that short stature is highly polygenic at the population level yet frequently oligogenic or monogenic at the level of the individual child, and that milder or atypical presentations of known skeletal dysplasias and syndromes are common among children currently classified as ISS 8 9 [10]. Copy-number and rare-variant analyses have similarly shown that genetic lesions detectable in "idiopathic" cohorts overlap with loci known from syndromic growth disorders 5 [11].

In parallel, the clinical work-up of short stature still rests on a small set of quantitative measurements: auxology, serum IGF-1 and IGFBP-3, and skeletal maturation assessed as bone age [12 13 14]. Each component has caveats: IGF-1 and IGFBP-3 have limited diagnostic sensitivity for growth hormone insufficiency [15], and bone-age readings, although informative about remaining growth potential, are method- and reference-dependent [14]. Therapeutically, recombinant GH is approved for ISS in some jurisdictions; meta-analytic evidence indicates modest average gains in adult height with considerable inter-individual variability, and age at treatment start and first-year responsiveness are major determinants of height outcome [16 17]. Which children benefit most, and whether molecular subtyping could refine prediction, remains unresolved.

A further practical obstacle is that much of the clinically relevant phenotype in short-stature care—family history, perinatal events, dysmorphic features, developmental concerns—is recorded in unstructured free text. Semi-automated approaches to phenotyping from electronic medical records have matured in other

domains [18], but their application to pediatric growth clinics is limited. Finally, many centers now hold sequencing data generated for clinical or research purposes, yet analytical reuse of such data in small, single-center cohorts is rarely reported with appropriate caveats.

Against this background, we performed an integrative, exploratory analysis of children evaluated at a single pediatric clinic, combining (i) structured clinical measurements, (ii) phenotype categories extracted from free-text chief-complaint notes, and (iii) pre-existing sample-level coding-variant matrices. Our aims were to characterize the clinical contrast between ISS (height SDS < − 2) and normal-height clinic children (height SDS > − 1); to quantify the relationship between growth severity and bone-age delay; to describe coding-variant burden and generate a candidate, ISS-enriched gene list; to explore phenotype-defined ISS subgroups; and to situate the resulting candidates within known protein-interaction and therapeutic contexts. We deliberately retain all analyses at the level of hypothesis generation: the cohort is small, the comparison group is clinic-ascertained rather than population-based, the variant matrices were not filtered for allele frequency or predicted pathogenicity, and gene-level tests were not corrected for genome-wide multiplicity.

2. Materials and Methods

2.1 Study design and ethics

This was a single-center, retrospective, exploratory analysis of children evaluated at the pediatric clinic of the Second Affiliated Hospital of Xi'an Jiaotong University. The study protocol was reviewed and approved by the ethics committee of the Second Affiliated Hospital of Xi'an Jiaotong University (No. 2024156). Written informed consent and/or waiver-of-consent arrangements. All data were analyzed in de-identified form. The study is reported as hypothesis-generating; no result was used to change clinical management.

2.2 Study population and grouping

Children evaluated at the clinic with available height SDS and clinical notes were eligible. ISS was defined operationally as height SDS < − 2, following the height threshold used in the ISS consensus statement 1; this is a clinic-based operational definition and does not imply that each child underwent the complete guideline-level exclusionary work-up (e.g., formal exclusion of GH deficiency, small-for-gestational-age status, or syndromic diagnoses) required for a strict ISS diagnosis 2 3. A normal-height comparison group was defined as height SDS > − 1. Children with − 2 ≤ height SDS ≤ − 1 were excluded from the primary contrast so that the two groups were separated by at least one SDS unit. The normal-height arm consisted of children attending the same clinic for non–short-stature reasons; it is not a population-based healthy control sample.

The clinical set comprised 25 ISS and 22 normal-height children. Sample-level coding-variant matrices were available for a subset of 22 ISS and 7 normal-height children. Analyses that required both height

SDS and a given laboratory or radiographic variable used complete cases for that variable; per-test sample sizes are reported throughout.

2.3 Clinical variables

Height SDS was taken from the clinical record. Serum IGF-1 and IGFBP-3 were detected by the MAGLUMI 800 auto-chemiluminescence immunoassay analyzer (Snibe, China). Bone age was read clinically, and bone-age difference (BAD) was computed as bone age minus chronological age in years; negative BAD indicates delayed skeletal maturation. Structured binary flags in the record (facial abnormality, cardiac anomaly, urinogenital anomaly, skeletal dysplasia, motor delay, intellectual disability, genital abnormalities) were retained as recorded.

2.4 Phenotype extraction from free-text notes

Free-text chief-complaint notes were mapped to controlled English phenotype terms and to 17 higher-level categories (growth, facial, skeletal/limb, neurodevelopment, sex development, family history, perinatal, and others) using an LLM-assisted extraction workflow. This workflow was conceptually motivated by semi-automated electronic-health-record phenotyping approaches such as PheCAP [18], but PheCAP itself was not used. Category presence was coded as 1 if any term in that category was extracted for a child. Term-level flags used for ISS subgrouping included dysmorphic facies, intellectual disability, familial short stature, and preterm birth. Extraction was performed once on all notes and then joined to the analytic tables; the identical mapping was applied to ISS and normal-height children.

2.5 Sequencing data and variant matrices

Sequencing data had been processed previously into sample-level coding-variant matrices, with variants classified as missense, nonsense, nonstop, frameshift insertion, frameshift deletion, in-frame insertion, in-frame deletion, or translation start-site change (eight class-level columns). No additional allele-frequency, inheritance, or pathogenicity filter was applied in this analysis; consequently, a gene is scored as “mutated” for a class if any variant of that class is present, and gene-level tests reflect variant presence rather than rare, likely-pathogenic alleles. Total mutation burden was defined as the sum over all eight variant-class columns; high-impact burden was the sum of frameshift, nonsense, nonstop, and start-loss variants, excluding in-frame indels and missense variants.

2.6 ISS-enriched genes and pathway annotation

For each variant class, genes were tested for a difference in mutation frequency between sequenced ISS and sequenced normal-height children using Fisher’s exact test on a 2 × 2 table of mutated versus non-mutated samples. Genes with nominal $P < 0.05$ and a higher frequency in ISS ($\Delta > 0$) were called ISS-enriched. The union of ISS-enriched genes across classes was subjected to Gene Ontology (GO) over-representation analysis using clusterProfiler with org.Hs.eg.db (ontology “ALL”) [19], referencing the GO resource [20]. P values were left unadjusted at the enrichment call; terms were retained at $q < 0.2$. Because no genome-wide multiple-testing correction was applied to the gene-level scans, these analyses are exploratory.

2.7 Bone-age analyses

BAD was compared between ISS and normal-height children with the two-sided Wilcoxon rank-sum test. Spearman's rank correlation was used for height SDS versus BAD (within ISS children with BAD, and in the sequenced subset with BAD) and for high-impact burden versus BAD. Among sequenced children with available BAD, each gene mutated in at least three samples was tested for a BAD difference between mutated and non-mutated carriers (Wilcoxon rank-sum test). Genes with nominal $P < 0.05$ were carried forward to GO analysis as in Section 2.6.

2.8 ISS phenotype subgroups

Within sequenced ISS children (n = 22), a phenotype term was eligible for gene-level contrast if at least three children were positive and at least three were negative. Eligible terms were dysmorphic facies (6 vs 16), intellectual disability (4 vs 18), familial short stature (4 vs 18), and preterm birth (3 vs 19). Subgroup-enriched genes were defined as in Section 2.6, comparing Present versus Absent, and BAD was compared between Present and Absent within ISS using the Wilcoxon test.

2.9 Protein–protein interaction network, known ISS genes, and drug shortlist

ISS-enriched genes, BAD-associated genes, and SRCAP were used as seed genes for a STRING query (Homo sapiens, combined score ≥ 0.40) [21]; the network was restricted to edges connecting seed genes, and node degree was used as a simple hub score. Candidate drugs were compiled from the growth-plate and GH–IGF literature and from prior SRCAP docking work; this panel is a literature/docking-derived shortlist and does not represent a statistical enrichment test against an interaction database such as DGIdb. Missense frequencies of a pre-specified ISS-related gene list (SHOX, ACAN, NPR2, FGFR3, GHRHR, GH1, GHR, STAT5B, IGF1, IGFALS, IGF1R, PTPN11, COL2A1, IHH, SRCAP) were compared between sequenced ISS and normal-height children with Fisher's exact test when both groups had non-missing calls.

2.10 Statistical analysis

Two-sided Wilcoxon rank-sum tests were used for continuous variables and Fisher's exact tests for binary frequencies. Correlations used Spearman's rank coefficient (r_s). Sample sizes are reported with each test. No genome-wide multiple-testing correction was applied to gene-level Fisher or Wilcoxon scans; $P < 0.05$ is treated as a nominal discovery threshold, not as evidence of genome-wide significance. Analyses were performed in R (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Analytic cohort

Forty-seven children entered the primary contrast: 25 with ISS (14 girls, 11 boys; median age 8.0 years) and 22 with normal height (6 girls, 16 boys; median age 11.4 years) (Table 1). Age did not differ significantly between groups (P = 0.16). A post hoc Fisher's exact test did not show a significant sex-distribution difference (P = 0.076), although the ISS group contained a higher proportion of girls. Median height SDS was − 2.3 in ISS versus − 0.2 in the normal-height group (P = 4.1×10^{-9}), as required by the grouping rule (Fig. 1A). Sequencing matrices were available for 22 ISS and 7 normal-height children; this sequenced subset underpins all gene-level analyses (Table 2).

Table 1
Clinical and extracted-phenotype characteristics of the study cohort (primary contrast).

Characteristic	**ISS (n = 25)**	**Normal height (n = 22)**	**P value**
Girls / boys, n	14 / 11	6 / 16	0.076
Age, years, median	8.0	11.4	0.16
Height SDS, median	−2.3	−0.2	4.1×10^{-9}
IGF-1, median, ng/ml	101.4	351.2	1.2×10^{-5}
IGFBP-3, median,ug/ml	3.19	5.20	9.6×10^{-5}
Bone-age difference, years, median	−2.1	0.1	2.9×10^{-8}
Growth-related terms extracted, n/N	24/25	7/22	4.2×10^{-6}
Facial category, %	40.0	9.1	0.020
Skeletal/limb category, %	32.0	4.5	0.025
Neurodevelopmental category, %	44.0	13.6	0.029
Hematologic category, %	0	18.2	0.041
Skin category, %	0	18.2	0.041
Two-sided Wilcoxon rank-sum test (continuous) or Fisher's exact test (categorical).			

Table 2
Overview of sequencing-based and enrichment analyses (sequenced subset).

Analysis	ISS	Normal height	Statistic / note
Sequenced samples, n	22	7	Subset of the clinical cohort
Total coding-variant burden, median	7,897	8,975	Wilcoxon P = 0.0044
High-impact burden, median □	408	568	Wilcoxon P = 0.0047
ISS-enriched gene–class tests / unique genes	37 / 35	—	Fisher's exact nominal P < 0.05, higher frequency in ISS
Examples of top ISS-enriched genes	FAM83G ($P = 9.9 \times 10^{-4}$), FHAD1, IRX5 (both P = 0.0015), CEP128 (P = 0.0022)	—	Mostly missense
GO, ISS-enriched 35-gene union	Chromatin-remodeling complexes (EP400, BPTF); DNA-related catalytic activity	—	Exploratory; smallest q = 0.14
Sequenced subset with BAD, n	26 (both groups)	—	—
Height SDS vs BAD	$r_s = 0.75$, $P = 9.1 \times 10^{-6}$	—	Spearman, n = 26
High-impact burden vs BAD	$r_s = 0.25$, P = 0.22	—	Spearman, n = 26
BAD-associated gene–class tests / unique genes	212 / 207	—	Wilcoxon nominal P < 0.05
GO, BAD-associated genes	Lipid-binding and acyl-CoA–related molecular functions	—	Exploratory; smallest q = 0.061
Subgroup-enriched genes, n	Facies 69; intellectual disability 47; familial short stature 71; preterm birth 45	—	Present vs Absent, nominal P < 0.05
Familial short stature vs BAD (within ISS)	Present vs Absent	—	Wilcoxon P = 0.0058 (sequenced); P = 0.009 (clinical set)
STRING seed network	14 nodes, 12 edges; hub SPAG17 (degree 4)	—	Combined score ≥ 0.40, seed–seed edges

□ Sum of frameshift, nonsense, nonstop, and start-loss variants.

Analysis	ISS	Normal height	Statistic / note
SRCAP edges	SRCAP–EP400 score 0.90; SRCAP–BPTF score 0.47	—	—
SRCAP missense frequency	0/22	4/7	Fisher's exact P = 0.0015; not interpretable as causal or protective (unfiltered matrices)

 Sum of frameshift, nonsense, nonstop, and start-loss variants.

3.2 Clinical and extracted phenotypes

Circulating GH–IGF axis markers and skeletal maturation differed markedly between the groups (Table 1; Fig. 1B–D). Median IGF-1 was 101.4 ng/ml in ISS versus 351.2 ng/ml in normal-height children ($P = 1.2 \times 10^{-5}$), and median IGFBP-3 was 3.19 ug/ml versus 5.20 ug/ml ($P = 9.6 \times 10^{-5}$). Bone age was substantially more delayed in ISS: median BAD was − 2.1 versus 0.1 years ($P = 2.9 \times 10^{-8}$). IGF-1 and IGFBP-3 were missing in one ISS and two normal-height children, and BAD was missing in two ISS and two normal-height children; complete cases were used per test.

Phenotype categories extracted from free-text notes recapitulated the expected clinical picture (Fig. 1E). Growth-related terms were present in 24/25 ISS versus 7/22 normal-height children ($P = 4.2 \times 10^{-6}$). Facial (40.0% vs 9.1%; P = 0.020), skeletal/limb (32.0% vs 4.5%; P = 0.025), and neurodevelopmental (44.0% vs 13.6%; P = 0.029) categories were also more frequent in ISS. Conversely, hematologic and skin categories were recorded more often in the normal-height clinic group (both 18.2% vs 0%; P = 0.041), consistent with that arm comprising clinic attendees with their own reasons for referral rather than a healthy reference sample.

Within ISS children who had BAD measurements (n = 23), more severe height deficit tracked with more delayed skeletal maturation: height SDS correlated positively with BAD (Spearman $r_s = 0.51$; P = 0.012; Fig. 1F). Among extracted terms, familial short stature was associated with greater bone-age delay (mean BAD − 2.53 years when present [n = 4] vs − 2.03 years when absent; P = 0.009), whereas dysmorphic facies, intellectual disability, and preterm birth showed no significant association with BAD in this ISS set (Fig. 1G).

3.3 Mutation burden and ISS-enriched genes

Among sequenced children (22 ISS, 7 normal height), total coding-variant counts and high-impact counts were lower in ISS than in the normal-height set (total median 7,897 vs 8,975, P = 0.0044; high-impact median 408 vs 568, P = 0.0047; Fig. 2A,B). The distribution of variant classes was otherwise broadly similar between groups (Fig. 2C). Given that the normal-height sequenced set is small (n = 7) and clinic-ascertained—and that matrices were not filtered for rarity or predicted pathogenicity—this burden difference should not be read as a deficit of rare disease alleles in ISS.

Thirty-seven gene–class tests met the nominal threshold of $P < 0.05$ with higher frequency in ISS, corresponding to 35 unique genes (Fig. 2D); most were missense. The smallest P values included FAM83G in-frame insertion ($P = 9.9 \times 10^{-4}$), FHAD1 and IRX5 missense (both $P = 0.0015$), and CEP128 missense ($P = 0.0022$). GO over-representation analysis of the 35-gene union highlighted chromatin-remodeling complexes (including EP400 and BPTF) and DNA-related catalytic activity; however, the smallest q value was 0.14, so no term survives a conventional false-discovery-rate cutoff, and these annotations are hypothesis-generating only (Fig. 2E; Table 2).

3.4 Bone-age delay in the sequenced subset

The clinical bone-age contrast persisted in the sequenced subset: BAD remained lower in sequenced ISS than in sequenced normal-height children (Fig. 3A). Height SDS and BAD were strongly correlated among sequenced children with BAD ($n = 26$; $r_s = 0.75$; $P = 9.1 \times 10^{-6}$; Fig. 3B), reinforcing the coupling between statural severity and skeletal delay observed in the full clinical set. In contrast, high-impact mutation count was not correlated with BAD ($r_s = 0.25$; $P = 0.22$; Fig. 3C), providing no support for a simple dosage relationship between aggregate disruptive variants and maturational delay.

Gene-level scans against BAD yielded 212 gene–class tests with a BAD difference at nominal $P < 0.05$ (207 unique genes), dominated by missense variants (Fig. 3D). GO analysis of this larger list returned lipid-binding and acyl-CoA–related molecular functions at nominal $P < 0.001$, with q values down to 0.061 (Fig. 3E). Because these gene lists are long relative to the sample size and arise from unfiltered matrices, they should be treated strictly as hypothesis-generating.

3.5 ISS phenotype subgroups

Within the 22 sequenced ISS children, the number of genes with higher mutation frequency in the Present arm was 69 for dysmorphic facies (6 vs 16), 47 for intellectual disability (4 vs 18), 71 for familial short stature (4 vs 18), and 45 for preterm birth (3 vs 19) (Fig. 4A). Familial short stature remained the only subgroup showing a BAD difference (Present vs Absent, $P = 0.0058$; Fig. 4B), in line with the analysis of the larger clinical ISS set.

GO analysis of familial-short-stature–enriched genes pointed to BMP/activin signaling (GDF2, ACVRL1, GATA6, FSTL3; nominal $P \approx 2 \times 10^{-4}$). Intellectual-disability–enriched genes produced the largest GO table (280 terms); ten terms—all cellular-component annotations such as nuclear membrane, nuclear envelope, and sarcoplasmic reticulum—reached $q < 0.05$, whereas the nominated phospholipid biosynthetic processes (ETNK2, CHKA) remained nominally significant only ($q \approx 0.19$). Dysmorphic-facies enrichment yielded only two GO terms, and preterm birth yielded 37 terms, none with $q < 0.05$; the composite figure displays the dysmorphic-facies and familial-short-stature panels (Fig. 4C,D).

3.6 Interaction network and known ISS genes

The seed-only STRING network (combined score ≥ 0.40, seed–seed edges retained) contained 12 edges among 14 genes (Fig. 5A). SPAG17 had the highest degree (4), acting as the principal hub (Fig. 5B).

SRCAP connected to EP400 (combined score 0.90) and BPTF (0.47), placing it alongside the chromatin-remodeling proteins that had emerged among ISS-enriched genes.

A shortlist of literature-derived growth-pathway drugs and prior SRCAP–PKCθ docking candidates is shown in Fig. 5C; this panel is a curated shortlist from the literature and docking work, not a statistical enrichment test against an interaction database, and is presented only to indicate translational directions.

Among the pre-specified ISS-related genes with missense calls, SRCAP missense variants were absent in sequenced ISS (0/22) and present in 4/7 sequenced normal-height children (P = 0.0015; Fig. 5D). Because the matrices were not filtered for rare or predicted-pathogenic alleles and the normal-height sequenced set comprises only seven clinic attendees, this contrast demonstrates neither SRCAP loss of function in ISS nor a protective effect in normal-height children. Other listed genes with calls in both groups (ACAN, GHRHR, GHR, GH1, IGFALS, IGF1R) did not differ at P < 0.05, and several genes had no calls in the missense matrix.

4. Discussion

In this single-center integrative analysis, children operationally defined as ISS (height SDS < − 2) differed from normal-height clinic children (height SDS > − 1) across three complementary data layers. Clinically, ISS children showed lower IGF-1 and IGFBP-3 and markedly delayed bone age, and the severity of the height deficit tracked the degree of skeletal delay both in the full clinical set and, more strongly, in the sequenced subset. Phenotypically, categories extracted from free-text notes—facial, skeletal/limb, and neurodevelopmental—were enriched in ISS, echoing reports that children labeled “idiopathic” frequently carry subtle additional features 7 8 [10]. Genomically, exploratory scans of unfiltered coding-variant matrices nominated 35 ISS-enriched genes whose GO annotation converged on chromatin-remodeling complexes, and a seed-gene network placed SRCAP adjacent to two such remodelers, EP400 and BPTF.

The coupling between height SDS and BAD ($r_s = 0.51$ within ISS; $r_s = 0.75$ in the sequenced subset) is consistent with the established clinical role of bone age as an integrator of growth tempo and remaining growth potential [14]. Two observations sharpen this picture. First, within ISS, familial short stature was the only extracted phenotype associated with greater bone-age delay (clinical set P = 0.009; sequenced subset P = 0.0058), suggesting that in this cohort the familial label may preferentially capture children with a constitutional/delayed-maturation component, although a growth-plate genetic contribution cannot be excluded—an interpretation that must remain tentative given only four affected children. Second, high-impact variant burden was not correlated with BAD (P = 0.22), arguing against a simple relationship in which more disruptive variants per genome produce more maturational delay. If a genetic contribution to bone-age tempo exists here, it is more likely gene-specific than burden-wide, which is the logic behind the BAD-associated gene scan—although the 207-gene output of that scan is far too large, relative to 26 samples, to support any individual gene claim.

The most coherent theme across the exploratory gene lists was chromatin regulation. The ISS-enriched union contained EP400 and BPTF, core subunits of the EP400/TIP60 and NURF remodeling complexes, respectively, and GO terms for chromatin-remodeling complexes topped the annotation of this list (albeit at q = 0.14). SRCAP, the catalytic ATPase of the H2A.Z-depositing SRCAP complex, was added as a seed because heterozygous truncating SRCAP variants cause Floating–Harbor syndrome, in which short stature and delayed bone age are cardinal features [22 23]. In the seed-only STRING network, SRCAP was linked directly to EP400 (score 0.90) and BPTF (0.47), consistent with the shared H2A.Z-exchange biology of these complexes [24 25 26].

Chromatin regulation is a plausible—though unproven—mechanistic bridge to growth biology. Growth-plate chondrogenesis is exquisitely sensitive to transcriptional and epigenetic control [27 28], and BPTF has an established structural role in reading H3K4me3 marks at active promoters [25]. We therefore view the convergence of ISS-enriched genes on remodeling complexes as a prioritization signal for replication, not as evidence of mechanism.

The SRCAP missense contrast (0/22 ISS vs 4/7 normal height; P = 0.0015) requires particular restraint. Because the variant matrices were not filtered for allele frequency or predicted pathogenicity, “missense present” simply means that some missense call of unknown functional consequence exists. The direction of the contrast—fewer SRCAP missense calls in ISS—is the opposite of what a naive loss-of-function hypothesis would predict, and with only seven sequenced normal-height children the estimate is fragile. It should not be interpreted as evidence that SRCAP variation causes ISS, nor as evidence of a protective effect; it is reported for completeness and transparency.

Subgroup analyses, although limited to contrasts with at least three children per arm, generated coherent leads. Familial-short-stature–enriched genes annotated to BMP/activin signaling (GDF2, ACVRL1, GATA6, FSTL3), a pathway with established roles in chondrogenesis and growth-plate maintenance [28 29]; this direction is also consistent with the recognized contribution of growth-plate gene variants to familial short stature in treated cohorts [10]. Intellectual-disability–enriched genes produced the largest GO table, but its $q < 0.05$ terms were cellular-component annotations (nuclear membrane, nuclear envelope, sarcoplasmic reticulum) rather than the nominated phospholipid biosynthetic processes (ETNK2, CHKA; $q \approx 0.19$); given that neurodevelopmental phenotypes were themselves enriched in the ISS clinical set, this subgroup may warrant targeted follow-up in larger cohorts, and its GO output is a candidate for supplementary presentation.

None of the genomic findings is practice-changing. The clinically usable messages are conventional but worth restating with numbers: in this clinic population, ISS was accompanied by substantially lower IGF-1/IGFBP-3 and by a median bone-age delay of about two years, and simple extracted features (family history, facial, skeletal, neurodevelopmental) stratified the cohort in ways that mirror what genetic studies of short stature have shown at the molecular level 4 7 [10]. Structured capture of these features—rather than reliance on free text—would make such stratification routine. Established pharmacotherapy for growth disorders (GH in ISS [16 17]; vosoritide in achondroplasia as a proof of principle for growth-

plate–targeted therapy [30]; recombinant IGF-1 in severe primary IGF-1 deficiency [31]) illustrates that pathway-directed treatment is feasible once a mechanism is defined, which is precisely the step this exploratory analysis cannot take. The drug panel in Fig. 5C is accordingly presented as a literature- and docking-derived shortlist, not as an enrichment result or a treatment recommendation.

Several limitations bound the interpretation of every result. First, this is a single-center, retrospective, exploratory study of clinic-ascertained children; selection and referral biases are unavoidable, and the findings are hypothesis-generating. Second, ISS was defined operationally by the height SDS < − 2 threshold 1; a complete guideline-level exclusion of GH deficiency, small-for-gestational-age status, and syndromic diagnoses was not verified case by case, so the ISS arm may include children with identifiable causes. Third, the normal-height group comprised children attending the same clinic for other reasons—evidenced by their higher rates of hematologic and skin phenotypes—and is not a healthy population control. Fourth, the sequenced normal-height set contains only seven children, rendering all genomic contrasts (including the SRCAP observation) statistically fragile. Fifth, the variant matrices were not filtered for allele frequency, zygosity, inheritance, or predicted pathogenicity; “mutation” denotes presence of any variant of a class, and the mutation-burden differences should not be read as differences in rare deleterious alleles. Sixth, gene-level Fisher and Wilcoxon scans used a nominal $P < 0.05$ threshold without genome-wide multiple-testing correction, and the GO analyses are explicitly exploratory (ISS-enriched list: smallest $q = 0.14$; BAD-associated list: smallest $q = 0.061$). Seventh, free-text phenotype extraction depends on note content and quality, and extraction was performed once without a formal validation sample. Finally, subgroup contrasts involved as few as three children per arm, and the composite figures present only selected GO panels.

5. Conclusions

In a single-center cohort defined by an operational height-SDS threshold, ISS was accompanied by lower IGF-1 and IGFBP-3, pronounced bone-age delay tracking with height deficit, and enrichment of facial, skeletal/limb, and neurodevelopmental phenotypes extracted from clinical notes. Familial short stature identified the subgroup with the greatest skeletal delay. Exploratory scans of unfiltered coding-variant matrices nominated chromatin-remodeling genes (including EP400, BPTF, and network neighbor SRCAP) and subgroup-specific pathways (BMP/activin signaling; phospholipid biosynthesis) as candidates for follow-up. These signals are hypothesis-generating only; replication in larger, well-phenotyped cohorts with rarity- and pathogenicity-filtered variant calls is required before any mechanistic or clinical inference can be drawn.

Abbreviations

BAD
bone-age difference (bone age minus chronological age)
BMP
bone morphogenetic protein

DGIdb
Drug–Gene Interaction database
FDR
false discovery rate
GH
growth hormone
GO
Gene Ontology
IGF-1
insulin-like growth factor 1
IGFBP-3
IGF-binding protein 3
ISS
idiopathic short stature
PKCθ
protein kinase C theta
SDS
standard deviation score
STRING
Search Tool for the Retrieval of Interacting Genes/Proteins.

Declarations

Ethics approval and consent to participate. The study was approved by the institutional ethics committee of the Second Affiliated Hospital of Xi'an Jiaotong University (No. 2024156). This study was conducted in accordance with the guidelines of the Research Committee of Xi`an Jiaotong University and according to the Declaration of Helsinki. Written informed consent was obtained from all guardians of children participating in the study.

Clinical trial number: not applicable.

Consent for publication. All authors agreed to publish this research.

Availability of data and materials. De-identified analytic tables and variant matrices are available from the corresponding author upon reasonable request, subject to institutional and ethics-approval conditions. Original figures are provided as vector PDF files; PNG previews are embedded in this draft for review only.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. Yuesheng Liu designed and supervised experiments; Lijun Hao and Yifan Yang performed all the experiments; Biyao Lian and Wenxin Feng managed, analyzed, and ploted the data; Chunyan Yin and Lijun Hao organized and wrote the paper; Yuesheng Liu and Lijun Hao critically revised the manuscript. All authors reviewed the final version of the manuscript.

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Figures

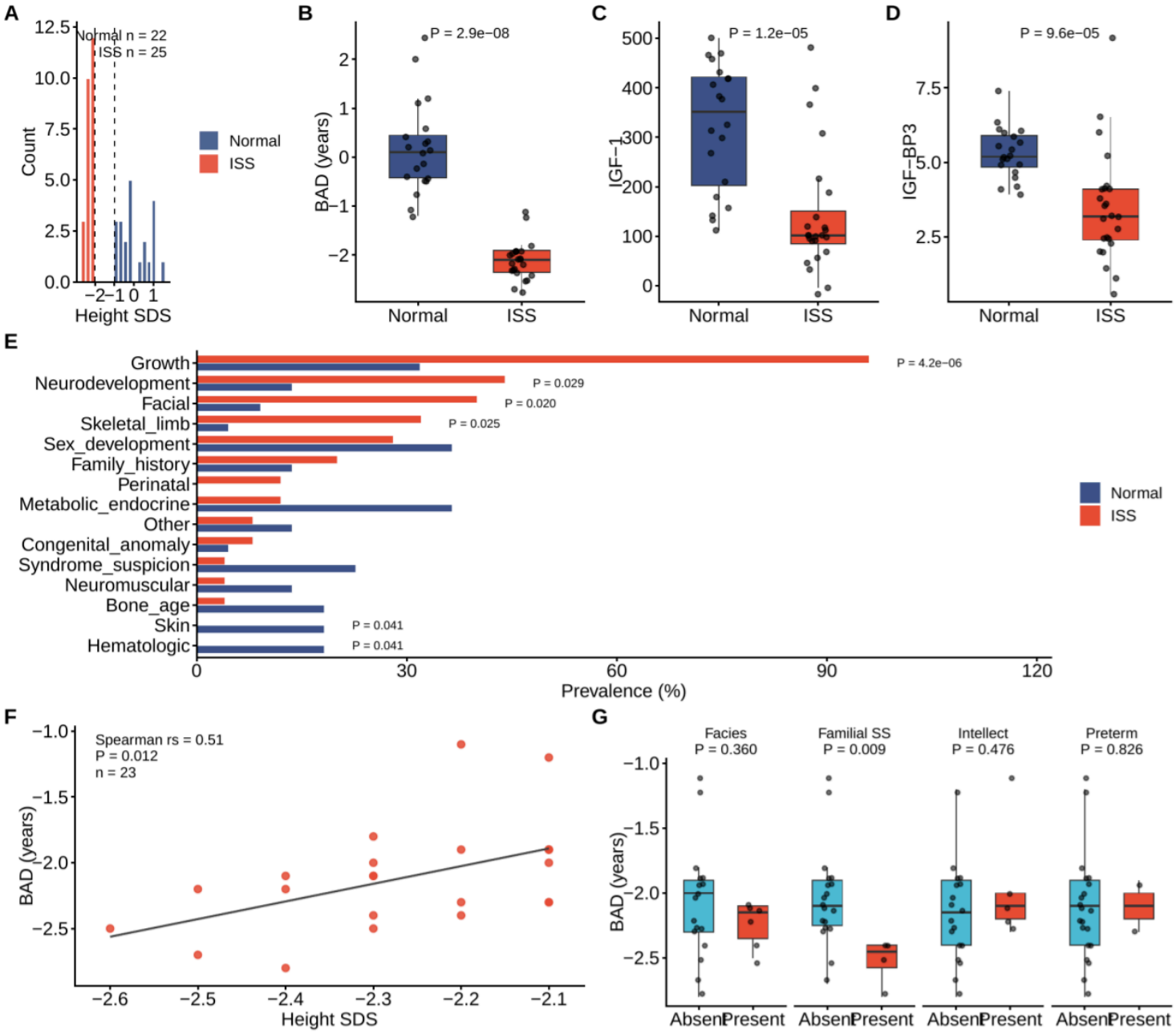


Figure 1

Clinical comparison of ISS (height SDS < −2) and normal height (SDS > −1). (A) Height SDS histogram. Dashed lines mark −2 and −1. Normal n = 22; ISS n = 25. (B–D) Bone-age difference (BAD, years), IGF-1, and IGFBP-3. Boxes show the median and interquartile range; points are individual children. P values are two-sided Wilcoxon tests. (E) Prevalence of LLM-extracted phenotype categories. P values (Fisher's exact test) are shown for categories with P < 0.05. (F) Height SDS versus BAD within ISS (n = 23). Spearman r_s, P, and n are printed on the panel. (G) BAD in ISS by presence or absence of four extracted terms. Facet P values are Wilcoxon tests.

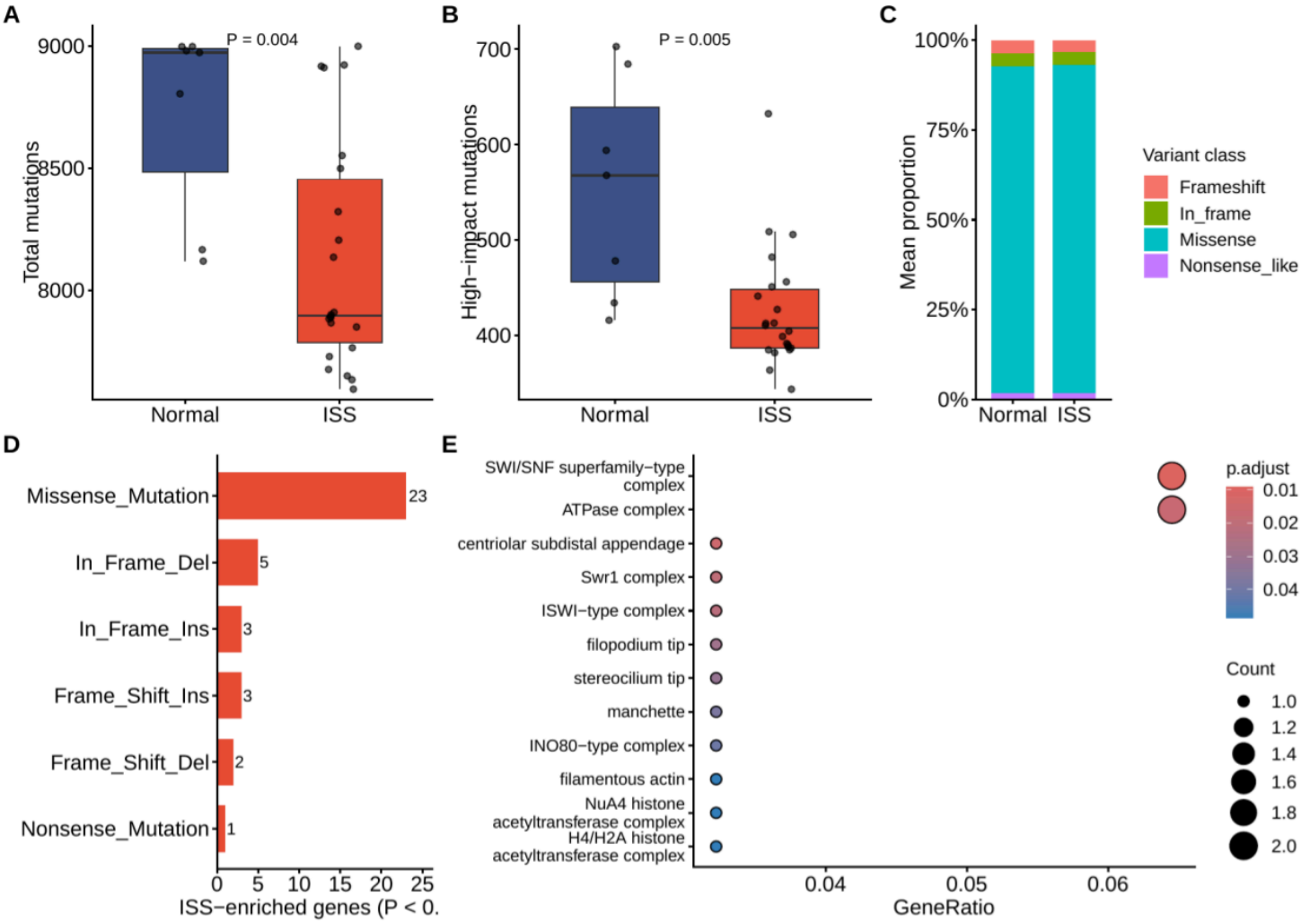


Figure 2

Mutation burden and ISS-enriched coding variants. (A, B) Total coding-variant count and high-impact count (frameshift, nonsense, nonstop, start-loss) in sequenced children (Normal n = 7; ISS n = 22). P values are Wilcoxon tests. (C) Mean proportion of variant classes by group. (D) Number of ISS-enriched genes (nominal P < 0.05 and higher frequency in ISS) by variant class. (E) GO terms for the 35-gene ISS-enriched union (exploratory; smallest q = 0.14).

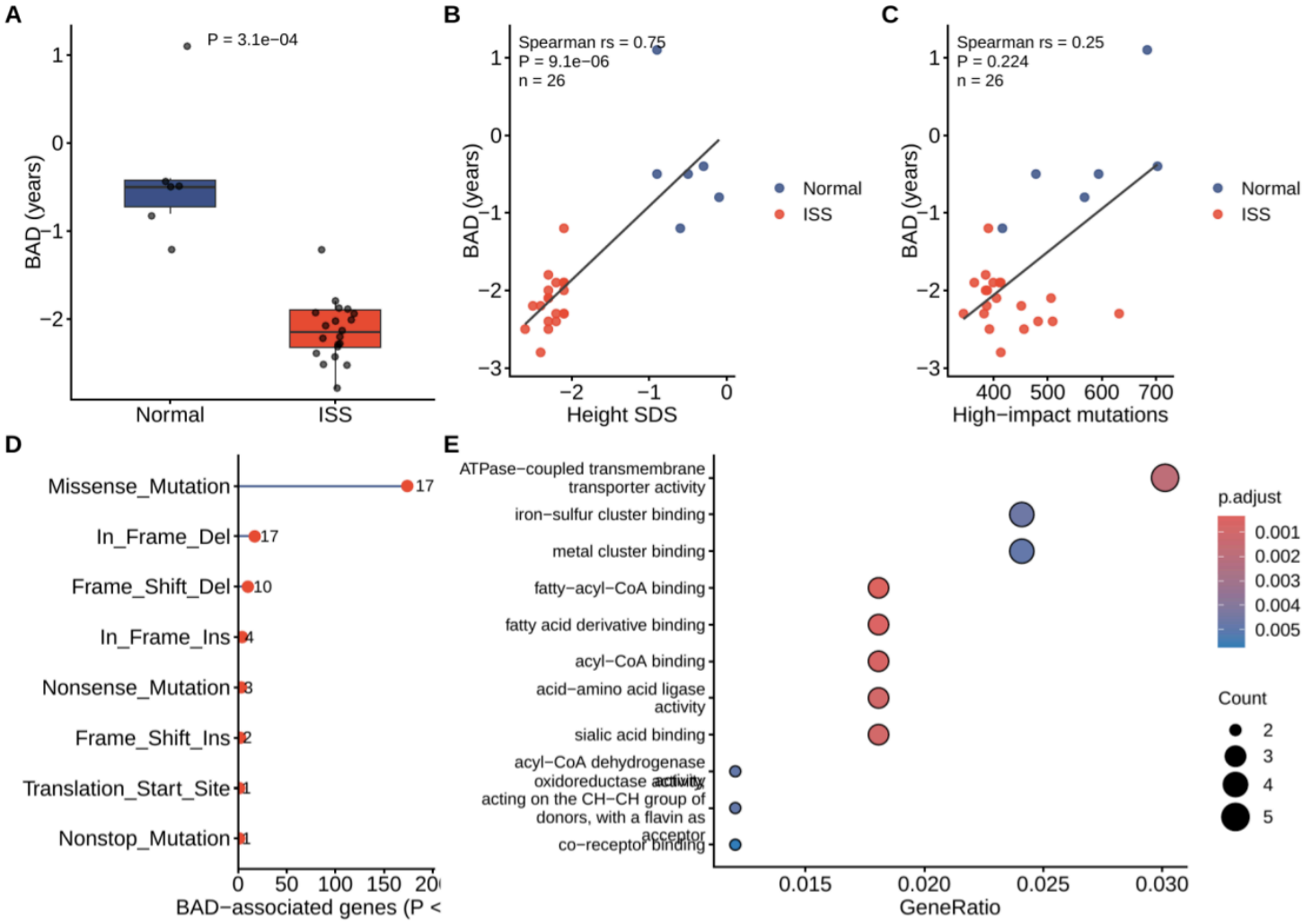


Figure 3

Bone-age difference in sequenced children.(A) BAD by group among sequenced children with BAD. (B) Height SDS versus BAD (n = 26; Spearman r_s = 0.75, P = 9.1 × 10^{-6}). (C) High-impact mutation count versus BAD (n = 26; r_s = 0.25, P = 0.22). (D) Count of genes associated with BAD at Wilcoxon nominal P < 0.05, by variant class. (E) GO terms for those genes (exploratory). Note for authors: the colorbar scale in the source panel does not match the text q-values (smallest q = 0.061); panel to be checked and regenerated before submission.

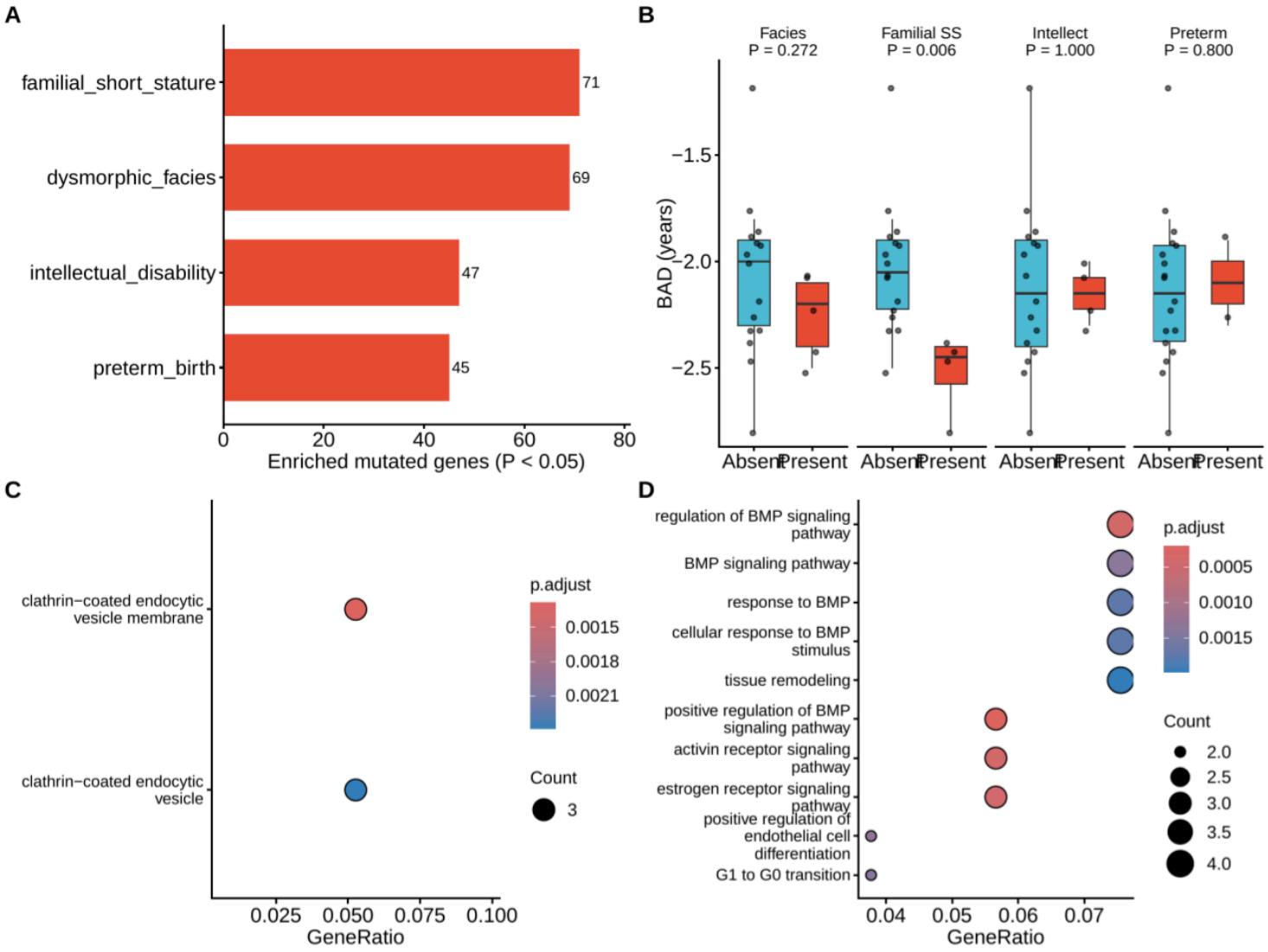


Figure 4

Sequenced ISS subgrouped by extracted phenotypes. (A) Number of genes with higher mutation frequency in the Present arm (nominal P < 0.05). (B) BAD in sequenced ISS by phenotype status; facet P values are Wilcoxon tests. (C, D) GO terms for dysmorphic-facies–enriched and familial-short-stature–enriched genes, respectively (exploratory).

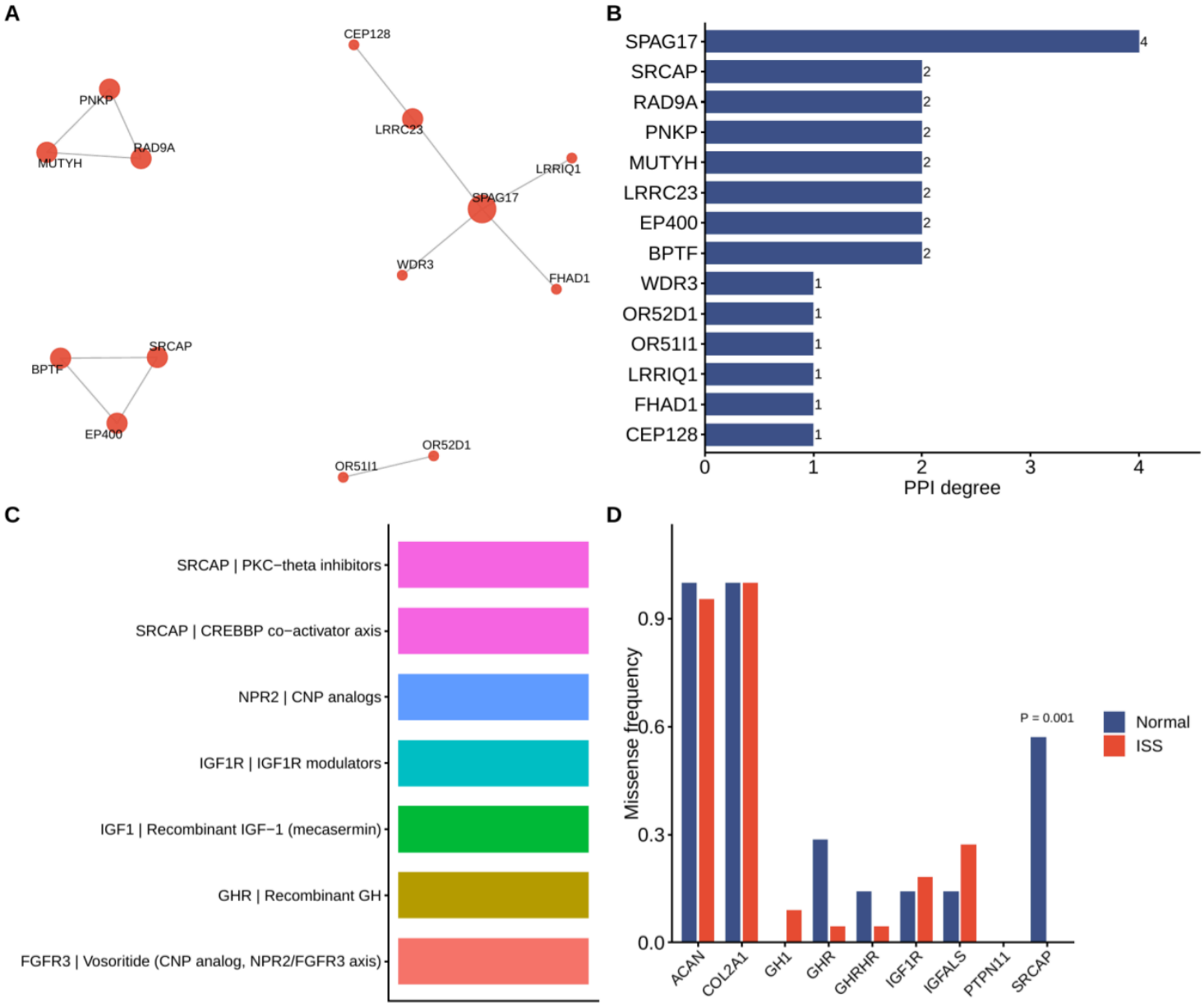


Figure 5

Seed-gene network and known ISS loci.(A) STRING interactions among seed genes (combined score ≥ 0.40), restricted to seed–seed edges. (B) Node degree for the same network. (C) Literature- and docking-based drug/gene pairs (a curated shortlist, not a statistical enrichment test). (D) Missense frequency of pre-specified ISS-related genes in sequenced ISS versus sequenced normal-height children. P is shown when Fisher's exact test P < 0.05 (SRCAP).