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Genotype–Phenotype Spectrum of Overgrowth Syndromes in Polish and Lithuanian Cohorts

Kamilė Šiaurytė-Jurgelėnė [1,*], Karolis Baronas [1], Aušra Matulevičienė [1], Beata Aleksiūnienė [1], Monika Mozerė [1], Natalia Wawrusiewicz-Kurylonek [2], Beata Stasiewicz-Jarocka [2], Barbara Panasiuk [2], Alireza Tafazoli [3], Błażej Marciniak [4], Joanna Michalska-Madej [4,5], Algirdas Utkus [1] and Renata Posmyk [2]

[1] Department of Human and Medical Genetics, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, LT-08661 Vilnius, Lithuania
[2] Department of Medical Genetics, Medical University in Bialystok, 15-269 Bialystok, Poland
[3] Division of Research and Development, MyEngene Canada Inc., Calgary, AB T2P 0N7, Canada
[4] Centre for Digital Biology and Biomedical Science-Biobank Łódź, Faculty of Environmental Protection, University of Lodz, 91-402 Lodz, Poland
[5] BioMedChem Doctoral School of the University of Lodz and Lodz Institutes of the Polish Academy of Science, University of Lodz, 90-237 Lodz, Poland
* Correspondence: kamile.siauryte@mf.vu.lt

Abstract

Background/Objectives: Overgrowth syndromes (OGSs) are a heterogeneous group of rare conditions marked by excessive growth and variable neurodevelopmental and systemic features. They are broadly classified into generalized overgrowth (GOG) and segmental overgrowth (SOG), which differ in clinical presentation and genetic mechanisms. However, many cases remain genetically unresolved, especially in underrepresented populations. Methods: We analyzed a binational cohort of patients from Poland and Lithuania with suspected OGSs, using detailed clinical evaluation and exome sequencing (ES) from blood-derived DNA. Variants were interpreted using current clinical guidelines, and phenotypes were compared between the GOG and SOG groups. Results: Patients with GOG more often showed developmental delay, intellectual disability, neurological issues, and joint laxity, while vascular malformations were more common in patients with SOG. Dysmorphic features appeared earlier in the SOG group, but became more pronounced over time in the GOG group. Genetic analysis led to the identification of definitive diagnoses in 25% of individuals. Pathogenic variants were identified in the genes involved in chromatin regulation and epigenetic control (*DNMT3A*, *EED*, *KMT5B*, *CHD3*, and *NSD1*), along with novel variants expanding the known spectrum. Conclusions: These findings provide valuable insights into the genetic architecture of OGSs in underrepresented Central and North-Eastern European populations, supporting the development of region-specific diagnostic and surveillance strategies.

Keywords: overgrowth syndromes; generalized overgrowth; segmental overgrowth; exome sequencing; novel genetic variants; epigenetics; neurodevelopmental disorders; rare diseases

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1. Introduction

Overgrowth syndromes (OGSs) are a highly heterogeneous group of rare disorders united by a shared clinical hallmark-excessive growth. This umbrella term encompasses conditions characterized by either generalized overgrowth (GOG) or segmental overgrowth (SOG). In most cases, abnormal growth patterns are detectable prenatally, although postnatal onset has also been reported [1]. GOG is defined by a proportional increase in height,

weight, and/or head circumference by at least two standard deviations (SD) above the mean for age- and sex-matched peers [2]. In contrast, SOG refers to disproportionate enlargement of a specific body region compared to the contralateral anatomical counterpart [3].

Although individually rare, OGSs collectively affect approximately 1 in 10,000 individuals [2] and are associated with several comorbidities, including an increased predisposition to pediatric neoplasia [4]. Advances in next-generation sequencing and multi-omics technologies have significantly improved diagnostic yield and expanded our understanding of the molecular basis of these conditions. Nevertheless, a considerable proportion of clinically diagnosed OGS cases remain genetically unresolved. A detection rate of approximately 30 to 50% has been reported [5,6]. This diagnostic gap limits the implementation of precision medicine approaches, particularly in syndromes with tumor predisposition, where timely molecular confirmation is critical for risk stratification and management.

The genetic architecture of OGSs is complex and reflects interactions between germline and somatic variants, epigenetic regulation, and dysregulated cellular growth pathways. A significant subset of generalized overgrowth disorders arises from constitutional (germline) variants causing monogenic conditions, including Sotos syndrome, Weaver syndrome, Primrose syndrome, Malan syndrome, and Cantu syndrome [3,7]. These disorders are typically inherited in an autosomal dominant manner and are caused by pathogenic variants in genes such as *NSD1*, *EZH2*, *ZBTB20*, *NFIX*, and *ABCC9* [8–12]. Despite being associated with clinically distinct phenotypes, these genes share common functional characteristics, as most encode transcriptional or epigenetic regulators involved in gene expression during embryonic and early postnatal development. Disruption of these pathways commonly results in macrocephaly, somatic overgrowth, neurodevelopmental impairment, and craniofacial dysmorphism—core features of GOG.

In contrast, SOG is most often associated with postzygotic somatic mosaic variants arising during embryogenesis and leading to a patchy distribution of affected tissues. This mechanism explains the regional nature of the phenotype and, in many cases, the absence of significant intellectual disability. Key genes implicated in these disorders include *PIK3CA*, *AKT1*, and *PTEN*, which are central components of the PI3K/AKT/mTOR signaling pathway—a critical regulator of cellular proliferation, growth, and survival [13,14]. Activating variants in *PIK3CA* underlie the PIK3CA-Related Overgrowth Spectrum [15], while somatic variants in *AKT1* cause Proteus syndrome [16]. Germline or mosaic *PTEN* variants are associated with disorders such as Cowden syndrome [17]. Together, these conditions highlight the pivotal role of dysregulated growth signaling in the pathogenesis of SOG.

While monogenic variants represent a major causative mechanism in the pathogenesis of specific syndromes, structural genomic alterations also contribute to overgrowth phenotypes. Chromosomal conditions often associated with generalized overgrowth include translocation 2q37, 3q13.31 deletion, 4p16.3 duplication, 5q35.3 deletion, and others [7]. In addition, submicroscopic copy number variants identified in affected individuals suggest that subtle genomic imbalances may account for a subset of currently unclassified OGS cases [18–20]. These findings underscore the importance of comprehensive genomic approaches, such as chromosomal microarray and genome sequencing, in diagnostic evaluation.

Epigenetic dysregulation represents another major pathogenic mechanism, particularly in OGSs associated with neurodevelopmental disorders and intellectual disability. Pathogenic variants in genes involved in chromatin remodeling and histone modification, such as *CHD8*, *DNMT3A*, *EED*, *EZH2*, *HIST1H1E*, *NSD1*, *SETD2*, *CDKN1C*, and *SUZ12*, disrupt transcriptional control during development and contribute to overgrowth phenotypes [3,21]. In addition to sequence-level pathogenic variants, disturbances in genomic

imprinting are significant in the pathogenesis of certain syndromes. For example, aberrant imprinting at chromosome 11p15 is a key molecular mechanism underlying Beckwith–Wiedemann syndrome and contributes to both somatic overgrowth and tumor predisposition [1]. Collectively, these findings highlight the convergence of genetic and epigenetic mechanisms driving abnormal growth.

Despite rapid progress in genomic medicine, global reference databases remain unbalanced in their population representation. Polish, Lithuanian, and other North-Eastern European populations are significantly underrepresented in large-scale genomic resources, such as gnomAD, the 1000 Genomes Project, and the UK Biobank. As a result, allele frequency estimates and variant classifications are often derived predominantly from Western European ancestry cohorts, limiting their interpretability in Central and Eastern European populations [22,23]. Recent studies have demonstrated the presence of population-specific and medically relevant variants in Polish and Lithuanian cohorts that are absent or underrepresented in broader European datasets [24,25]. This disparity represents the need for regionally focused genomic studies to improve variant interpretation and clinical applicability.

To address this gap, we established a binational collaboration between Lithuania and Poland—the Genetic Background of Overgrowth Syndromes in Polish and Lithuanian Populations: Basis for Rapid Genetic Testing to Prevent Neoplasias (GOSPL) project. This study presents the first comprehensive genomic analysis of OGSs in Polish and Lithuanian populations, reporting diagnostic outcomes, novel variants, and population-specific findings in affected individuals.

2. Materials and Methods

2.1. Study Cohort and Controls

Patients were recruited from Vilnius University Santaros Klinikos (Lithuania), the Medical University of Bialystok Clinical Hospital (Poland), and additional Clinical Genetics Centers in Poland. A total of 120 unrelated individuals of European ancestry with suspected overgrowth syndromes were included. The patients were classified into generalized overgrowth (GOG) and segmental overgrowth (SOG) groups based on clinical evaluation. Inclusion criteria comprised either segmental overgrowth or at least two of three anthropometric measurements (height, weight, and head circumference) above +2 SD. Clinical data were collected through a standardized clinical questionnaire on pregnancy history, prenatal and postnatal development, anthropometric measurements, dysmorphic features, and other clinical characteristics. Phenotypic assessment was performed by clinical geneticists. The control data used for this study were provided by the Data Access Committee of the Biobank Lab, Department of Oncobiology and Epigenetics, University of Lodz, in the European Genome–Phenome Archive database (accessed at https://ega-archive.org, accessed on 15 November 2024, accession number: EGAD50000000130) and consisted of 412 controls.

2.2. Statistical Analysis

The statistical analysis was conducted using Microsoft Excel and RStudio (v. 2026.05.0) with the R Commander package. For continuous variables, the normality assumption was checked using the Shapiro–Wilk test. For normally distributed variables, Student's *t*-test was performed to compare means; otherwise, the Mann–Whitney U (Wilcoxon) test was used to compare medians. Categorical variables were compared with Chi-square or Fisher's exact test based on the expected frequencies. Differences were deemed to be statistically significant when the p-value < 0.05.

2.3. Sequencing and Annotation

Genomic DNA samples from the Lithuanian cohort ($n = 33$) and Polish cohort ($n = 87$) underwent exome sequencing (ES) performed using CeGaT GmbH (Tübingen, Germany) on the Illumina NovaSeq 6000 platform (Illumina, San Francisco, CA, USA) and Twist enrichment kit (Twist Bioscience, San Francisco, CA, USA). All variant call files (VCFs) delivered by the sequencing provider were mapped or lifted over to the hg19/GRCh37 reference genome, using CrossMap (v. 0.7.0) software where necessary. Quality control and variant filtering were performed using an in-house pipeline with GATK (v. 4.0.5.1) and/or BCFTools (v. 1.10) software, applying a minimum coverage threshold of 20×. All filtered variant call files (VCFs) were subsequently annotated using ANNOVAR (version dated: 2025-03-02).

2.4. Variant Analysis

Following variant annotation, the variants were screened against ClinVar (accessed 14 January 2026) to identify previously reported pathogenic or likely pathogenic (P/LP) variants. These variants were manually reviewed to assess the supporting evidence and their consistency with the patients' clinical phenotypes.

The variants not classified as P/LP in ClinVar underwent further prioritization. The variants with a minor allele frequency (MAF) > 1% in gnomAD or detected in the control dataset ($n = 412$; Section 2.1) were excluded from further consideration. The missense variants were prioritized using SIFT (<0.05), PolyPhen-2 HDIV (>0.5), and CADD (Phred > 15), requiring all three thresholds to be met. The splice-site variants were evaluated using available splicing predictions, including dbscSNV ADA and RF scores. The stop-gain and frameshift variants were assessed based on their predicted molecular consequences and potential loss of function, supported by CADD, GERP++, and ALoFT predictions where applicable.

Candidate variants were further prioritized according to established gene–disease associations, expected inheritance patterns, and concordance with the patients' clinical phenotypes. The prioritized variants were subsequently classified according to ACMG/AMP guidelines, considering population frequency, molecular consequences, available functional and segregation evidence, and computational predictions (PP3 where appropriate). *De novo* occurrence was considered when parental data were available. Final classifications were assigned following manual review of the available evidence.

3. Results

3.1. Cohort Characteristics

The research cohort included 120 unrelated, non-consanguineous individuals of Lithuanian and Polish descent, comprising 50 females (41.7%) and 70 males (58.3%), with a median age of 63 years. Based on clinical assessment, the participants were classified into two groups: generalized overgrowth (GOG, 72/120, 60.0%) and segmental overgrowth (SOG, 48/120, 40.0%). Clinical and demographic characteristics, as well as p-values, are summarized in Appendix A, Table A1. Developmental delay in the neonatal period was significantly more frequent in the GOG group (68.1%) compared to the SOG group (27.1%, $p < 0.001$). Similarly, intellectual disability was more prevalent in the GOG group (65.3% vs. 14.6%, $p < 0.001$), as were other neurological disorders (51.4% vs. 31.3%, $p = 0.029$) and joint laxity (36.1% vs. 18.8%, $p = 0.040$). Overgrowth parameters were also more commonly observed in the GOG group, including height > +2 SD (72.2% vs. 35.4%, $p < 0.001$), weight > +2 SD (66.7% vs. 27.1%, $p < 0.001$), and head circumference > +2 SD (52.8% vs. 18.8%, $p < 0.001$). In addition, morphological anomalies at the time of evaluation were more frequent in the GOG group, affecting the

craniofacial region overall (80.6% vs. 58.3%, $p = 0.008$), as well as specific areas, such as the eyes (68.1% vs. 39.6%, $p = 0.002$), mouth (68.1% vs. 31.3%, $p < 0.001$), and ears (63.9% vs. 37.5%, $p = 0.005$). In contrast, dysmorphic features at the neonatal period were significantly more prevalent in the SOG group (22.2% vs. 47.9%, $p = 0.003$), as were vascular abnormalities (13.9% vs. 31.3%, $p = 0.022$). Family history of hemihypertrophy was also more commonly reported in the SOG group compared to the GOG group (1.4% vs. 10.4%, $p = 0.037$).

3.2. Diagnostic Yield

Genetic analysis led to the identification of definitive diagnoses in 25% (30/120) of individuals. A summary of all diagnoses established in this cohort is provided in Table 1. The identified conditions were separated into two groups. The majority of diagnoses (60.0%, $n = 18$) were overgrowth spectrum disorders, while 12 spanned multiple disease categories, including neurodevelopmental, endocrine, hereditary cancer disorders, and skeletal conditions. Out of the identified OGSs, 17 were associated with GOG and one with SOG. The diagnostic yield was 24% in the GOG group and 2% in the SOG group, respectively. The most diagnosed overgrowth condition was Sotos syndrome (five cases), with Tatton–Brown–Rahman syndrome coming a close second (four cases).

Table 1. The spectrum of the diagnosed disorders in the study cohort. The data are presented as the number of cases. MIM number—Online Mendelian Inheritance in Man database number, AD—autosomal dominant, XLR—X-linked recessive, GOG—generalized overgrowth, SOG—segmental overgrowth, OGSs—overgrowth syndromes, het—heterozygous, hem—hemizygous, P—pathogenic, LP—likely pathogenic, and VUS—variant of uncertain significance. Summary information for each section of the table is shown in bold.

Disorder, Inheritance	MIM Number	Gene	No. of Cases	Variant	Zygosity	ClinVar
Overgrowth spectrum			**18/120**			
GOG			**17/72**			
Cohen–Gibson syndrome, AD	#617561	*EED*	1	c.706A>G (p.Arg236Gly)	het	n.a.
Cowden syndrome, type 1, AD	#158350	*PTEN*	1	c.182A>G (p.His61Arg)	het	LP
Lujan–Fryns syndrome, XLR	#309520	*MED12*	1	c.6345_6347del (p.Gln2115del)	hem	VUS
Malan syndrome, AD	#614753	*NFIX*	1	c.1001C>A ((p.Ser326*))	het	n.a.
Simpson–Golabi–Behmel syndrome, type 1, XLR	#312870	*GPC3*	2	c.1159C>T (p.Arg387*) c.(477_574)_(1054_1253)del	hem hem	P n.a.
Sotos syndrome, AD	#117550	*NSD1*	5	c.2954_2955del (p.Ser985Cysfs*25) c.5753_5754del (p.Tyr1918*) c.3904C>T (p.Gln1302*) c.5127G>A (p.Trp1709*) c.6454C>T (p.Arg2152*)	het het het het het	P P P P P
Tatton–Brown–Rahman syndrome, AD	#615879	*DNMT3A*	4	c.1711G>C (p.Ala571Pro) c.1711dup (p.Ala571Glyfs*7) c.1523T>C (p.Leu508Pro) c. 1916T>C (p.Leu639Pro)	het het het het	n.a. P P/LP LP
Weaver syndrome, AD	#277590	*EZH2*	2	c.2050C>T (p.Arg684Cys)	het	P
GOG diagnostic yield			**24%**			
SOG			**1/48**			

Table 1. *Cont.*

Disorder, Inheritance	MIM Number	Gene	No. of Cases	Variant	Zygosity	ClinVar
PIK3CA spectrum disorder, somatic	#606773	*PIK3CA*	**1**	c.1798G>A (p.Glu600Lys)	het	P
SOG diagnostic yield			**2%**			
Total OGS diagnostic yield			**15%**			
Other pathology			**12/120**			
Basal cell nevus syndrome, type 1, AD	#109400	*PTCH1*	1	c.290del (p.Asn97Thrfs*20)	het	P/LP
Brachydactyly, type B1, AD	#113000	*ROR2*	1	c.1624G>A (p.Asn97Thr)	het	VUS
Congenital hypothyroidism, type 6, AD	#614450	*THRA*	1	c.1207G>A (p.Glu403Lys)	het	P
Developmental delay with variable intellectual impairment and behavioral abnormalities, AD	#618430	*TCF20*	2	c.2594C>G (p.Ser865*) c.3119del (p.Ser1040*)	het	P/LP n.a.
Intellectual developmental disorder, type 42, AD	#616973	*GNB1*	1	g.(1787322_1787437)_(1806475_1806538)del	het	n.a.
Intellectual developmental disorder, type 51, AD	#617788	*KMT5B*	1	c.913T>C (p.Cys305Arg)	het	n.a
Intellectual developmental disorder, type 55, with seizures, AD	#617831	*NUS1*	1	c.662T>A (p.Leu221*)	het	n.a.
Intellectual developmental disorder, X-linked, type 93, XLR	#300659	*BRWD3*	1	c.151_152insG (p.Lys51Argfs*24)	hem	n.a.
Phelan–McDermid syndrome, AD	#606232	*SHANK3*	1	c.895G>A (p.Ala299Thr)	het	VUS
Pseudohypoparathyroidism, AD	#103580, #612462	*GNAS*	1	c.1006C>T (p.Arg336Trp)	het	P/LP
Snijders Blok–Campeau syndrome, AD	#618205	*CHD3*	1	c.3253-1G>T (p.?)	het	n.a.
Total diagnoses			**30/120**			
Total diagnostic yield			**25%**			

3.3. Novel Genetic Variants

A total of eight novel variants, previously unreported in population databases or the literature, were identified across eight genes in the studied cohort (see Table 2). The variants were detected in genes previously associated with either the overgrowth spectrum (*EED* and NFIX) or neurodevelopmental/other syndromic disorders (*BRWD3*, *CHD3*, *DNMT3A*, *KMT5B*, *NUS1*, and *TCF20*). Five variants were predicted to result in loss of function, including frameshift, nonsense, and splice-site alterations, while the other three were missense changes. According to ACMG criteria, five variants were classified as pathogenic, and three as likely pathogenic. Phenotypes/clinical features of these patients were consistent with the associated diagnoses. Photographs of the available patients with novel variants are presented in Figure 1. Each gene was affected in a single patient, with no recurrent variants observed. None of the detected variants were present in the control group.

Table 2. The novel variants identified in the study cohort. The variants are described according to HGVS nomenclature. ACMG classification was assigned according to current guidelines. ACMG—American College of Medical Genetics and Genomics classification, LP—likely pathogenic, VUS—variant of uncertain significance, AD—autosomal dominant, XLR—X-linked recessive, het—heterozygous, and hem—hemizygous.

Gene	Variant	Zygosity	No. of Reference Sequence	Variant Type	ACMG	Disorder, Inheritance	MIM Number
BRWD3	c.151_152insG (p.Lys51Argfs*24)	hem	NM_153252.5	Null variant (frameshift indel)	**P** (PVS1, PM2, PM6)	Intellectual developmental disorder, X-linked, type 93, XLR	#300659
CHD3	c.3253-1G>T (p.?)	het	NM_001005273.3	Null variant (acceptor site)	**P** (PVS1, PM2, PM6, PP4)	Snijders Blok–Campeau syndrome, AD	#618205
DNMT3A	c.1711G>C (p.Ala571Pro)	het	NM_175629.2	Missense	**LP** (PM1, PM2, PM6, PP3, PP4)	Tatton–Brown–Rahman syndrome, AD	#615879
EED	c.706A>G (p.Arg236Gly)	het	NM_003797.5	Missense	**LP** (PM1, PM2, PM5, PM6, PP3, PP4)	Cohen–Gibson syndrome, AD	#617561
KMT5B	c.913T>C (p.Cys305Arg)	het	NM_017635.5	Missense	**LP** (PM1, PM2, PM5, PM6, PP3)	Intellectual developmental disorder, type 51, AD	#617788
NFIX	c.1001C>A (p.Ser326*)	het	NM_001271043.2	Null variant (stop gain)	**P** (PVS1, PM2, PM6, PP4)	Malan syndrome, AD	#614753
NUS1	c.662T>A (p.Leu221*)	het	NM_138459.5	Null variant (stop gain)	**P** (PVS1, PM2, PM6)	Intellectual developmental disorder, type 55, with seizures, AD	#617831
TCF20	c.3119del (p.Ser1040*)	het	NM_005650.4	Null variant (frameshift indel)	**P** (PVS1, PM2, PM6)	Developmental delay with variable intellectual impairment and behavioral abnormalities, AD	#618430



Figure 1. Photographs of patients with novel genetic variants: (**a**) *BRWD3*:c.151_152insG, (**b**) *DNMT3A*: c.1711G>C, (**c**) *EED*:c.706A>G, (**d**) *NFIX:* c.1001C>A, (**e**) *NUS1*:c.662T>A, and (**f**) *TCF20*:c.3119del.

4. Discussion

4.1. Phenotypic Findings

The phenotypic findings in our cohort were largely consistent with the existing literature. Developmental delay in the neonatal period, intellectual disability, and neurological manifestations were significantly more prevalent in the generalized overgrowth (GOG) group. Intellectual disability of varying severity is a well-recognized feature across most generalized overgrowth syndromes, although borderline or low-normal cognitive function has also been reported [7]. In contrast, intellectual disability is less commonly associated with segmental overgrowth (SOG), as these conditions are not typically classified among overgrowth–intellectual disability (OGID) syndromes, which are also known to be associated with neurological manifestations, such as epilepsy [26]. Vascular malformations, a hallmark feature of segmental overgrowth disorders, were more frequently observed in the SOG group, in line with previous reports [27]. Similarly, joint laxity was more prevalent in the GOG cohort, which is consistent with its known association with syndromes such as Sotos, Weaver, and Tatton–Brown–Rahman syndromes. Notably, the presence of joint laxity in combination with tall stature may lead to clinical misclassification, for example, as Marfan syndrome [27].

Differences were observed in the presence of dysmorphic features during the neonatal period, with a higher prevalence in the SOG group. Segmental overgrowth may be present at birth in conditions such as PIK3CA-related overgrowth spectrum disorders [28], while in others, such as Proteus syndrome, it may develop later [29]. One possible explanation is that asymmetry, if present, is more readily apparent in the neonatal period than other phenotypic features. Although dysmorphic features can occur in both groups, they tend to become more pronounced and clinically recognizable in GOG syndromes as the phenotype evolves. At the time of evaluation, the distribution of phenotypic features aligned more closely with established clinical expectations, with the GOG group demonstrating a significantly higher prevalence of craniofacial anomalies, including abnormalities of the head, face, eyes, mouth, and ears. Many generalized overgrowth syndromes are characterized by distinctive and recognizable features, such as dolichocephaly and a pointed chin in Sotos syndrome, prominent horizontal eyebrows in Tatton–Brown–Rahman syndrome, macroglossia in Beckwith–Wiedemann syndrome, and preauricular pits or tags in Simpson–Golabi–Behmel syndrome [30].

Unexpectedly, familial hemihyperplasia was observed more frequently in the SOG group. This finding contrasts with the existing literature, which indicates that most segmental overgrowth conditions, including PIK3CA-related overgrowth spectrum and Proteus syndrome, arise sporadically due to postzygotic variants and are, therefore, associated with a low recurrence risk [27]. Nevertheless, rare familial cases have been reported and may account for this observation [31].

4.2. Diagnostic Yield

Recent studies report that the diagnostic yield of exome sequencing (ES) in heterogeneous rare disease cohorts ranges from approximately 30–40% [32,33]. In the present study, the lower overall diagnostic yield of 25% likely reflects methodological and cohort-specific factors. Thus, the true diagnostic rate in our cohort may be underestimated.

In the GOG cohort, the diagnostic yield for OGS conditions reached 24% (17/72), while detection rates of 30–50% in similar cohorts have been reported [5,6]. Beckwith–Wiedemann syndrome is the most common genetic overgrowth syndrome, with an estimated prevalence of approximately 1 in 10,340 individuals [29], followed by Sotos syndrome (1 in 14,000) [10]. While the relatively high number of Sotos syndrome cases ($n = 5$) identified in our GOG cohort is consistent with this epidemiological data, the absence of Beckwith–Wiedemann

syndrome (BWS) cases suggests a potential underrepresentation. This discrepancy may be explained by two factors. First, the molecular heterogeneity of BWS, which frequently involves epigenetic alterations or mosaic changes that are not reliably detected by standard ES approaches. Second, patient selection: BWS has a recognizable clinical phenotype and is typically investigated through first-line testing strategies, such as methylation analysis and single-gene testing. Consequently, the patients with a clinically suspected or already established diagnosis of BWS were generally not referred for ES and were, therefore, not included in this study, which enrolled only patients without a prior molecular diagnosis. In contrast, Tatton–Brown–Rahman syndrome emerged as the second most frequent diagnosis in our cohort ($n = 4$), despite being considered an ultra-rare condition with a reported prevalence of less than 1 in 1,000,000 [34,35].

Only one SOG patient received a diagnosis through the ES approach (diagnostic yield of 2%, 1/48). A key limitation in this case is the exclusive use of blood-derived gDNA, which significantly reduces sensitivity for detecting somatic mosaic variants. This is particularly relevant in the context of overgrowth disorders, where mosaicism plays a major pathogenic role, such as in SOG. Previous studies have demonstrated that diagnostic yield can be substantially improved through tiered testing strategies incorporating affected tissues, especially in patients with clinical features suggestive of PIK3CA-related overgrowth spectrum and associated vascular anomalies [36].

4.3. Novel Genetic Variants

Several novel variants were identified in our cohort, expanding the mutational spectrum of the associated disorders. The identified novel variants predominantly cluster into two major functional categories: epigenetic regulators involved in chromatin remodeling (*DNMT3A*, *EED*, *KMT5B*, and *CHD3*) and genes associated with transcriptional and neurodevelopmental processes (*TCF20*, *NFIX*, and *BRWD3*), with additional variants affecting cellular pathways, such as protein glycosylation (*NUS1*).

The *BRWD3* gene variant c.151_152insG is predicted to introduce a frameshift early in the coding sequence, leading to a premature termination codon and likely resulting in either a truncated protein or nonsense-mediated mRNA decay. This finding supports the notion that truncating variants represent a common pathogenic mechanism in X-linked intellectual developmental disorder 93 [37,38]. High expression of *BRWD3* in the brain explains the neurodevelopmental phenotype. Additionally, it has been suggested that *BRWD3* pathogenic variants may disrupt intracellular signaling pathways affecting cellular proliferation, which may explain the overgrowth features observed in affected individuals, such as macrocephaly and obesity [39].

The *CHD3* gene variant c.3253-1G>T affects the splice acceptor site of exon 25 and is predicted to result in aberrant splicing, potentially leading to exon skipping or activation of a cryptic splice site and the production of a truncated or functionally altered protein. Exon 25 lies within the central region of the gene, which encodes part of the ATP-dependent helicase domain of the CHD3 protein. *CHD3* variants also disrupt transcriptional programs controlling cell fate and growth, which may underlie overgrowth and macrocephaly [40]. Although the helicase domain has been proposed as a mutational hotspot [41,42], ClinVar reports only eight pathogenic or likely pathogenic splice-site variants in *CHD3*, with a single variant (c.2343+2T>C) in the helicase region and none reported between exons 24–25.

The identified *DNMT3A* missense gene variant NM_175629.2:c.1711G>C is located within the ADD domain, a key regulatory region that mediates histone recognition and proper targeting of the methyltransferase to chromatin [43]. The ADD domain, together with the upstream PWWP domain and downstream methyltransferase catalytic domain, is a well-known mutational hotspot, where most missense variants cluster [44]. The variant

c.1711G>C was also investigated in a functional study by Huang et al., which demonstrated reduced DNMT3A methyltransferase activity [45].

The *EED* gene variant c.706A>G, detected in a patient with Cohen–Gibson syndrome, is predicted to result in a missense substitution within this WD40 repeat region, which is critical for PRC2 assembly and function [46]. Previously reported pathogenic *EED* variants are predominantly missense changes clustering within the WD40 domain, highlighting this region as a functional hotspot. Only 13 pathogenic/likely pathogenic *EED* variants have been reported in the ClinVar database to date. Cohen–Gibson syndrome is closely related to Weaver syndrome, which is caused by variants in *EZH2*, another core PRC2 component, highlighting a shared pathogenic mechanism involving the disruption of epigenetic repression [47].

The detected *KMT5B* variant c.913T>C results in a missense change located inside the canonical SET catalytic domain, suggesting a strong likelihood of disrupting enzymatic activity and chromatin regulation. Missense variants were previously reported to also occur outside well-characterized domains, but are still thought to impair protein function through the disruption of regulatory regions or protein–protein interactions [48]. Even though pathogenic variants in *KMT5B* are associated with an autosomal dominant neurodevelopmental disorder 51, a tendency for taller stature has also been observed in these patients; thus, there have been considerations to classify this pathology as overgrowth syndrome [49].

Previously reported *NFIX* pathogenic missense variants associated with overgrowth phenotypes tend to cluster in the N-terminal DNA-binding domain (exon 2), whereas truncating variants and splice-site changes distributed across exons 6–10 are more often associated with the severe Marshall–Smith phenotype [50]. Our reported variant c.1001C>A in a patient with Malan syndrome phenotype lies outside the canonical missense hotspot in exon 2 but falls within exon 7, a region where truncating and splice-site variants associated with Marshall–Smith syndrome have been reported. Notably, this stop-gain variant is located within the Marshall–Smith mutational hotspot yet results in a Malan syndrome phenotype, suggesting that factors beyond variant location and type, such as the fate of the mutant transcript or the specific position of the premature stop codon, may contribute to phenotypic outcome.

The *NUS1* gene variant c.662T>A, located in exon 3, is predicted to result in early loss of function via nonsense-mediated mRNA decay. Previously reported *NUS1* variants include a heterogeneous spectrum of missense, frameshift, nonsense, and splice-site changes, distributed throughout the gene, rather than confined to a single hotspot [51]. Notably, both truncating and missense variants across the *NUS1* coding sequence have been associated with overlapping neurodevelopmental phenotypes, and no clear genotype–phenotype correlation has been established, supporting a shared pathogenic mechanism related to impaired dolichol biosynthesis rather than domain-specific effects [52].

The *TCF20* gene variant c.3119del is predicted to result in a frameshift and premature termination codon, leading to loss of normal protein function, most likely through nonsense-mediated decay. This supports a haploinsufficiency mechanism, which is well established for *TCF20*, a gene shown to be highly intolerant to loss-of-function variation [53]. Compared to previously reported variants, *TCF20* changes predominantly consist of loss-of-function variants, including nonsense and frameshift changes distributed throughout the gene, with relatively few missense variants described [53].

To summarize, our analysis further highlights epigenetic regulation as a major dysregulated biological process in individuals with overgrowth and intellectual disability. Previous studies have shown that variants in epigenetic regulator genes (*NSD1*, *EZH2*, *DNMT3A*, *CHD8*, *HIST1H1E*, and *EED*) account for up to 44% of OGID cases [6]. Consistent with this,

our findings demonstrate that variants in genes directly involved in epigenetic regulation comprised 50.0% (15/30) of diagnoses, including *NSD1*, *DNMT3A*, *EZH2*, *EED*, *CHD3*, *GNAS*, and *KMT5B*.

5. Conclusions

Our findings highlight distinct phenotypic patterns between GOG and SOG, and underscore the central role of epigenetic regulation and growth signaling pathways in OGS pathogenesis. This study expands the molecular spectrum of overgrowth syndromes and emphasizes the importance of incorporating tissue-specific testing strategies to improve diagnostic yield. Furthermore, it provides valuable insights into the genetic architecture of OGSs in underrepresented Central and North-Eastern European populations, supporting the development of region-specific diagnostic and surveillance strategies.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki (2013 revision) and was approved by the local Bioethics Committees (protocol no. 2022/5-1435-907, approved on 10 May 2022, Lithuania; APK.002.352.2021, Poland, approved on 24 June 2021).

Informed Consent Statement: Written informed consent was obtained from all participants or their legal guardians.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Conflicts of Interest: A.T. is an employee of MyEngene Canada Inc., a commercial company operating in the field of pharmacogenomics and precision medicine. MyEngene Canada Inc. had no role in the design of this study, collection or analysis of the data, interpretation of the results, preparation of this manuscript, or decision to submit this manuscript for publication. The other authors declare no conflicts of interest. The funders had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of this manuscript; or in the decision to publish the results.

Appendix A

Table A1. Clinical characteristics and phenotype summary. GOG—generalized overgrowth, SOG—segmental overgrowth, NC—natural conception, IVF—in vitro fertilization, wg—gestational weeks, OFC—occipitofrontal circumference, SD—standard deviation, and IQR—interquartile range. * In 1st or 2nd degree relatives. Title for each section of the table and statistically significant results (p-value < 0.05) are shown in bold.

Characteristics	GOG	SOG	p-Value
No. of subjects	72	48	NA
Female (%)	28 (38.9)	22 (45.8)	0.450
Male (%)	44 (61.1)	26 (54.2)	0.450
Median age (yrs) (IQR)	6.3 (11.8)	6.3 (8.8)	0.618
Pregnancy history			
NC (%)	71 (98.6)	46 (95.8)	0.563
IVF (%)	1 (1.4)	2 (4.25)	0.563
1st pregnancy (%)	37 (51.4)	22 (45.8)	0.551
Complicated pregnancy (%)	19 (26.4)	18 (37.5)	0.197
History of pregnancy loss (%)	20 (27.8)	8 (16.7)	0.159
Smoking (mother) (%)	6 (8.3)	5 (10.4)	0.698
Smoking (father) (%)	15 (20.8)	11 (22.9)	0.786
Family history			
Mean mother's age at child's birth (yrs) ± SD	28.9 ± 5.3	29.8 ± 4.6	0.336
Mean father's age at child's birth (yrs) ± SD	31.9 ± 5.6	32.2 ± 4.5	0.756
Mean mother's height (cm) ± SD	167.8 ± 7.8	167.1 ± 5.9	0.556
Mean father's height (cm) ± SD	182.5 ± 7.3	181.3 ± 7.5	0.403
Mean mother's weight (kg) ± SD	78.6 ± 19.5	73.6 ± 16.1	0.151
Mean father's weight (kg) ± SD	90.7 ± 14.6	89.2 ± 13.8	0.610
Family history * of intellectual disability (%)	15 (20.8)	12 (25.0)	0.592
Family history * of overgrowth (%)	22 (30.6)	10 (20.8)	0.238
Family history * of hemihypertrophy (%)	1 (1.4)	5 (10.4)	**0.037**
Family history * of cancer (%)	39 (54.2)	26 (54.2)	1
Prenatal period			
USG abnormalities 11–13 wg (%)	5 (6.9)	2 (4.2)	0.701
USG abnormalities 20 wg (%)	11 (15.3)	3 (6.3)	0.131
USG abnormalities 30 wg (%)	8 (11.1)	6 (12.5)	0.816
Neonatal period			
Median birth weight (g) (IQR)	3700.0 (887.5)	3800.0 (635.0)	0.451
Median birth length (cm) (IQR)	56.0 (4.5)	56.0 (5.5)	0.777
Median birth OFC (cm) (IQR)	36.0 (3.0)	35.0 (3.0)	0.158
Jaundice (%)	20 (27.8)	12 (25.0)	0.736
Hypoglycemia (%)	6 (8.3)	2 (4.2)	0.474
Poor feeding (%)	17 (23.6)	8 (16.7)	0.358
Hypotonia (%)	27 (37.5)	11 (22.9)	0.092
Dysmorphic features (%)	16 (22.2)	23 (47.9)	**0.003**
Congenital defects (%)	22 (30.6)	15 (31.3)	0.936
Developmental delay (%)	49 (68.1)	13 (27.1)	**<0.001**
Growth parameters (at the time of evaluation)			
Height > +2SD (%)	52 (72.2)	17 (35.4)	**<0.001**
Weight > +2SD (%)	48 (66.7)	13 (27.1)	**<0.001**
OFC > +2SD (%)	38 (52.8)	9 (18.8)	**<0.001**

Table A1. *Cont.*

Characteristics	GOG	SOG	*p*-Value
Other clinical features			
Tumors (%)	4 (5.6)	7 (14.6)	0.093
Cardiac anomalies (%)	20 (27.8)	10 (20.8)	0.389
Vascular abnormalities (%)	10 (13.9)	15 (31.3)	**0.022**
Pigmentary abnormalities (%)	18 (25.0)	10 (20.8)	0.597
Skeletal problems (%)	30 (41.7)	23 (47.9)	0.499
Neurological disorders (%)	37 (51.4)	15 (31.3)	**0.029**
Joint laxity (%)	26 (36.1)	9 (18.8)	**0.040**
Genitourinary anomalies (%)	22 (30.6)	8 (16.7)	0.085
Intellectual disability (%)	47 (65.3)	7 (14.6)	**<0.001**
Dysmorphic features			
Head/face (%)	58 (80.6)	28 (58.3)	**0.008**
Eye region (%)	49 (68.1)	19 (39.6)	**0.002**
Nose region (%)	30 (41.7)	12 (25.0)	0.061
Mouth region (%)	49 (68.1)	15 (31.3)	**<0.001**
Ear region (%)	46 (63.9)	18 (37.5)	**0.005**
Upper limbs (%)	34 (47.2)	20 (41.7)	0.549
Lower limbs (%)	41 (56.9)	25 (52.1)	0.600
Thorax (%)	28 (38.9)	12 (25.0)	0.114

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