Ultrasound Obstet Gynecol 2023; **62**: 361–368
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.26188.

Postnatal genetic and neurodevelopmental assessment in infants born at term with severely low birth weight of non-placental origin

M. F. PAZ Y MIÑO[1], M. PAUTA[2], E. MELER[1], I. MATAS[1], E. MAZARICO[1], A. CAMACHO[3], M. SEGURA[4], F. FIGUERAS[1,2,5] and A. BORRELL[1,2,5]

[1]*BCNatal - Barcelona Center for Maternal–Fetal and Neonatal Medicine, Hospital Clínic Barcelona and Hospital Sant Joan de Déu, Barcelona, Catalonia, Spain;* [2]*IDIBAPS, University of Barcelona, Fetal i+D Fetal Medicine Research, Barcelona, Catalonia, Spain;* [3]*Mental Health and Perinatal Nursing, Hospital Clínic Barcelona, Barcelona, Catalonia, Spain;* [4]*qGenomics, Esplugues de Llobregat, Catalonia, Spain;* [5]*Departament de Cirurgia i Especialitats Medicoquirúrgiques, Facultat de Medicina i Ciències de la Salut, Universitat de Barcelona, Barcelona, Catalonia, Spain*

KEYWORDS: exome sequencing; fetal growth restriction; FGR; genetic syndrome; intrauterine growth restriction; IUGR; neurodevelopmental disorder; prenatal diagnosis

CONTRIBUTION

What are the novel findings of this work?
Non-malformed severely small infants born at term with no clinical or Doppler signs of placental insufficiency have a high rate of monogenic syndromes and neurodevelopmental impairment during childhood.

What are the clinical implications of this work?
Prenatal counseling of parents with a severely growth-restricted fetus at term should consider the risk of a postnatal genetic syndrome and neurodevelopmental delay, although further studies are required to confirm these findings.

ABSTRACT

Objective *To determine the frequency of genetic syndromes and childhood neurodevelopmental impairment in non-malformed infants born at term with severely low birth weight and no evidence of placental insufficiency.*

Methods *This case series was constructed from the data of infants delivered at term between 2013 and 2018 with severely low birth weight, defined as birth weight more than 2.5 SD below the mean, with normal maternal and fetal Doppler (umbilical artery, fetal middle cerebral artery, cerebroplacental ratio and uterine artery) and no maternal hypertensive disorder during pregnancy or fetal structural anomaly on prenatal ultrasound examination. Clinical exome sequencing and copy number variation (CNV) analysis were performed using DNA extracted from the children's saliva. Cognitive and psychomotor development was evaluated using the Bayley Scales of Infant and Toddler Development, 3rd edition or the Wechsler Intelligence Scale for Children, 5th edition tests, according to the child's age at testing.*

Results *Among the 36 405 infants born within the study period, 274 (0.75%) had a birth weight below −2.5 SD, of whom 98 met the inclusion criteria. Among the 63 families contacted, seven (11%) reported a postnatal diagnosis of a genetic syndrome and a further 18 consented to participate in the study. Median gestational age at delivery was 38.0 (interquartile range (IQR), 37.3–38.5) weeks and median birth weight was 2020 (IQR, 1908–2248) g. All 18 children showed a normal result on clinical exome sequencing and CNV analysis, but six (33%) obtained a low score on neurodevelopmental testing.*

Conclusion *Non-malformed severely small term infants with no clinical or Doppler signs of placental insufficiency present a high rate of genetic syndromes and neurodevelopmental impairment during childhood.* © *2023 The Authors. Ultrasound in Obstetrics & Gynecology published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Small-for-gestational age (SGA) is a catch-all condition defined as a fetal weight below the 10th centile. As

Correspondence to: Dr A. Borrell, Hospital Clínic Maternitat, Sabino Arana 1, 08028 Barcelona, Catalonia, Spain (e-mail: aborrell@clinic.cat)

Accepted: 3 February 2023

a large proportion of SGA fetuses are constitutionally small and healthy, an international consensus has been established on the criteria to identify, within the overall SGA population, those with pathological fetal growth restriction (FGR) that are at higher risk of adverse perinatal outcome[1]. In those with severe smallness and no Doppler signs of placental dysfunction, particularly when the diagnosis is made early in the third trimester, amniocentesis for chromosomal microarray analysis (CMA) is recommended[2,3]. When fetal growth deviates extremely from the normal range, a very high prevalence of genetic syndromes, diagnosed during postnatal follow-up, has been reported[4].

Exome sequencing is a high-throughput sequencing technique used to determine the nucleotide sequence of exons. The exome, which is comprised of all exons within the genome, accounts for approximately 1% of the human genome and may explain 85% of Mendelian diseases[5]. The contribution of monogenic diseases to severe smallness with normal Doppler and fetal anatomy is still unknown.

Furthermore, severe smallness has been associated with impaired neurodevelopment in term infants, since FGR has been described as a risk factor for cerebral palsy[6], intellectual disability[7], attention deficit hyperactivity disorder (ADHD)[8] and autism spectrum disorder[9]. Although the association between SGA and abnormal neurodevelopment is better understood in preterm SGA infants, there is emerging evidence that term SGA infants are also at higher risk of adverse neurodevelopmental outcome; a meta-analysis showed that their standardized neurodevelopmental scores were 0.32 SD below those of normal-sized controls[10]. Therefore, whether severe smallness in term pregnancies without placental insufficiency or an underlying genetic disorder is associated with abnormal neurodevelopment remains largely unknown, and is a highly relevant issue in parental counseling.

The primary aim of this study was to assess the frequency of monogenic disorders in children born at term with a severely low birth weight and normal Doppler signs. Moreover, we aimed to estimate the frequency of neurodevelopmental impairment when a genetic etiology was ruled out among these severely growth-restricted fetuses.

METHODS

Study design, setting and participants

This was a study of non-malformed infants born at term with severely low birth weight and no antenatal evidence of placental insufficiency at two BCNatal centers (Hospital Clinic Barcelona and Hospital Sant Joan de Déu) in Barcelona, Spain, during a 5-year period (August 2013 to September 2018). The inclusion criteria were: (i) singleton pregnancy; (ii) born at term (defined as $\geq 37+0$ weeks according to first-trimester crown–rump length); (iii) birth weight more than 2.5 SD below the mean, according to local standards[11]; (iv) normal Doppler studies of the umbilical artery, fetal middle cerebral artery, cerebroplacental ratio and uterine artery at the final examination (usually within 1–2 weeks before delivery); (v) no maternal diagnosis of hypertensive disease; and (vi) no fetal structural or genetic anomalies or fetal infection diagnosed prenatally. In our center, a routine third-trimester scan is performed at 36–37 weeks in the general pregnant population and, when the estimated fetal weight lies below the 20th percentile, Doppler studies and serial ultrasound scans are scheduled weekly. Exclusion criteria were children aged between 3.5 and 6 years, because they fall outside the age range for neurodevelopmental assessment, and those with incomplete data in their medical records.

Written informed consent to participate was obtained from parents on behalf of their children enrolled in the study, which was approved by the Institutional Review Boards of the Hospital Clinic Barcelona (HCB/2018/0077) and Hospital Sant Joan de Déu (HSJD/2021/PIC132-21).

Construction of case series

First, electronic birth registries of the Hospital Clinic and the Hospital Sant Joan de Déu were screened to select those infants meeting the inclusion criteria. Parents with infants aged 2–3.5 years or 6–8 years were contacted by telephone to double-check that the necessary criteria were met and to invite them to participate in the study. Those who signed the informed consent were asked to complete a questionnaire on demographic characteristics and obstetric and pediatric history. Children and both parents were scheduled for a face-to-face interview during which pretest counseling was provided (parents were given the opportunity to choose whether they wished to be informed of incidental pathogenic variants) and neurodevelopment was assessed by a certified developmental psychologist (A.C.).

Exome sequencing and copy number variation analysis

Genomic DNA samples were extracted from the children's saliva using a specialized kit for DNA collection, stabilization and transportation (DNA Genotek, Ottawa, ON, Canada). Parental blood samples were also collected in order to extract DNA for subsequent segregation studies, if required. DNA quality was determined by optical density using a DeNovix DS-11 instrument (Wilmington, DE, USA). The human exome was enriched using the KAPA HyperExome assay (Roche NimbleGen, Pleasanton, CA, USA) and massively sequenced using NovaSeq 6000 equipment (Illumina, San Diego, CA, USA), as described previously[12]. The average coverage depth achieved along the target regions was 65–140 ×.

Sanger sequencing in the index case and parents was performed to confirm the inheritance pattern of the most promising variant candidates. Negative results were reviewed to search for possible causative genes and ensure correct coverage in the next-generation sequencing experiment. A multidisciplinary Clinical

Review Committee composed of six members[12] reviewed all candidate variants and negative results. This committee could request a review of the coverage of specific genes with negative results, expansion of the parental segregation analysis or the study of elder siblings or other members of the family. Copy number variation (CNV) detection was included in the bioinformatic pipeline using the ExomeDepth program (R software, Vienna, Austria), which is highly sensitive for the large rearrangements but may not detect single-exon alterations.

Neurodevelopmental assessment

The Bayley Scales of Infant and Toddler Development, 3rd edition (BSID-III) or Wechsler Intelligence Scale for Children, 5th edition (WISC-V) tests were used according to the infant's age at testing. The BSID-III is an extensive formal developmental assessment tool for diagnosing developmental delay in early childhood[13]. This test evaluates children aged between 1 and 42 months in five key developmental domains, of which three are essential and comprise a cognitive scale with 91 items, a language scale with 49 items in receptive and 48 in expressive communication and a motor scale with 66 items in fine motor and 72 in gross motor function. The test takes about 30 to 70 min to complete. Raw data were homogenized by standardizing all raw scores to a mean of 100 and SD of 15 based on an American population. Finally, the development of the child was classified according to the final score (with 95% CI) and the developmental age score in months[14]. The following cut-offs were used, as reported previously[14]: < 70, extremely low; 70–79, low; 80–89, low–average; 90–109, average; 110–119, average–high; 120–129, superior; ≥ 130, very superior.

The WISC-V[15] evaluates cognitive performance in children aged between 6 and 16 years based on five factors: verbal comprehension, visual spatial, fluid reasoning, working memory and processing speed. Cut-offs were defined as above[16]. Percentiles were calculated using a normative American population.

Statistical analysis

Descriptive statistics were calculated. The Shapiro–Wilk test was used to assess whether variables were normally distributed. When P was > 0.05, normality of distribution was evaluated further by visual assessment of stem-and-leaf plots. Non-normally distributed variables are expressed as median (interquartile range (IQR)), while normally distributed variables are expressed as mean ± SD. Categorical variables are expressed as n (%) with 95% CI. For non-normally distributed variables, the Mann–Whitney U-test was used to determine significant differences between groups. Data were analyzed using SPSS software (Chicago, IL, USA); two-sided P of < 0.05 was considered to indicate statistical significance.

RESULTS

Participants and recruitment

Among the 36 405 term infants born during the study period, 274 (0.75%) had a birth weight below −2.5 SD. Of those, 176 infants were excluded because of abnormal prenatal Doppler studies ($n = 113$), a maternal diagnosis of a hypertensive disorder of pregnancy ($n = 24$), child's age between 3.5 and 6 years ($n = 21$) or a prenatal diagnosis of a congenital defect ($n = 18$) (Figure 1). A further 35 couples could not be contacted. Among the 63 couples contacted, seven (11%) reported a postnatal diagnosis of a genetic syndrome and 18 (29%) agreed to undergo both exome sequencing and neurodevelopmental testing.

Characteristics of participants

Among the 18 infants who underwent clinical exome sequencing, the median maternal age was 35.6 (IQR, 33.8–37.7) years (Table 1) and the median gestational age at delivery was 38.0 (IQR, 37.3–38.5) weeks (Table 2). The median birth weight was 2020 (IQR, 1908–2248) g, while median birth-weight Z-score was −2.8 (IQR, −3.2 to −2.6) and median birth-weight percentile was 0.23 (IQR, 0.04–0.38) (Table 2). Seven pregnant women had undergone invasive diagnostic testing with normal karyotyping ($n = 2$) or CMA ($n = 5$) results.

Genetic findings

Seven (11%) of the 63 mothers/couples contacted reported a postnatally diagnosed genetic syndrome, of which five were Mendelian monogenic and two were epigenetic. There were two cases of Cockayne syndrome (including one of cerebro-oculo-facio-skeletal syndrome), one of short stature, microcephaly and endocrine dysfunction syndrome, one of Renpenning syndrome, one of Noonan syndrome, one of Silver–Russell syndrome and one of Prader–Willi syndrome (Table 3). The median birth weight was 1902 (IQR, 1650–2190) g, which was not significantly different from that of non-syndromic children ($P = 0.495$). Clinical exome sequencing and CNV analysis in the 18 children without a postnatally diagnosed genetic syndrome yielded normal results in all cases.

Neurodevelopmental assessment

The BSID-III test was performed in seven children whose ages ranged from 23 months and 26 days to 42 months and 6 days, and the WISC-V test was performed in 11 children with ages ranging from 6 years to 8 years and 6 months. Among the 18 children in whom postnatal neurodevelopment was assessed, the mean score for cognitive function was 105 ± 19.

None of the seven children assessed using the BSID-III attained a score below 80 (i.e. below the cut-off for average neurodevelopment[14,17]) on any of the three scales

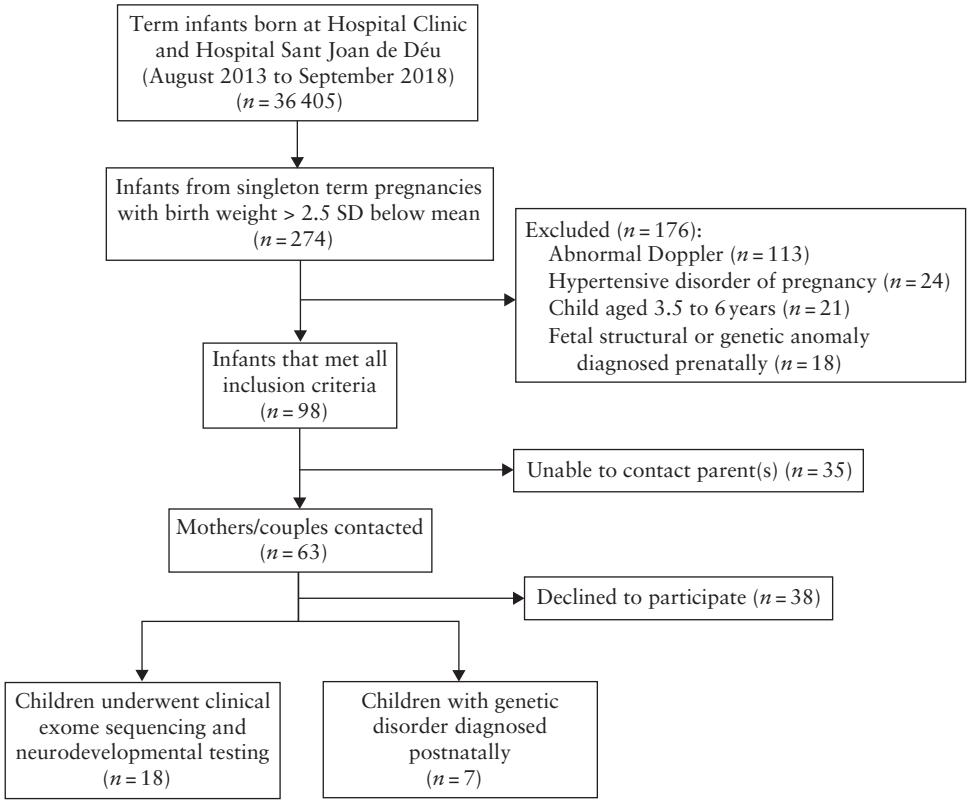

Figure 1 Flowchart summarizing inclusion in study of children born at term with severely low birth weight.

Table 1 Maternal and pregnancy characteristics of 18 cases that underwent clinical exome sequencing and neurodevelopmental testing

Characteristic	*Value*
Maternal age (years)	35.6 (33.8–37.7)
Ethnicity	
Caucasian	16 (88.9 (65–98))
Latin American	1 (5.6 (1.4–27))
Asian	1 (5.6 (1.4–27))
Maternal education level	
Primary school	2 (11.1 (1.4–34))
Secondary school	2 (11.1 (1.4–34))
Technical school	4 (22.2 (6.4–48))
High school	1 (5.6 (1.4–27))
College	9 (50.0 (26–74))
Parity	2 (2–2)
Nulliparous	7 (38.9 (17–64))
History of fetal loss*	4 (22.2 (6.4–48))
History of fetal growth restriction*	4 (22.2 (6.4–48))
History of pre-eclampsia*	0 (0)
Use of ART	1 (5.6 (1.4–27))
Smoking in pregnancy	3 (16.7 (3.5–41))
Use of alcohol in pregnancy	2 (11.1 (1.4–34))
Use of recreational drugs in pregnancy	0 (0)
Prenatal infection	0 (0)
Gestational diabetes mellitus	2 (11.1 (1.4–34))
Prenatal genetic testing	7 (38.9 (17–64))
Karyotype	2 (11.1 (1.4–34))
Chromosomal microarray analysis	5 (27.8 (9.7–54))

Data are given as median (interquartile range), *n* (% (95% CI)) or *n* (%). *In previous pregnancy. ART, assisted reproductive techniques.

Table 2 Birth and neonatal characteristics of 18 cases that underwent clinical exome sequencing and neurodevelopmental testing

Characteristic	*Value*
Cesarean section	8 (44.4 (21–69))
GA at birth (weeks)	38.0 (37.3–38.5)
Birth weight (g)	2020 (1908–2248)
Birth-weight *Z*-score	−2.8 (−3.2 to −2.6)
Birth-weight percentile	0.23 (0.04–0.38)
Female infant sex	13 (72.2 (47–90))
Umbilical artery pH	7.25 (7.23–7.31)
Neonatal acidosis	0 (0)
Apgar score at 5 min	9 (9–9)
Apgar score at 10 min	10 (10–10)
Apgar score < 7 at 5 min	0 (0)
Abnormal metabolic screening	0 (0)
Breastfeeding*	13/17 (76.5 (46–90))
Duration (months)*	5 (4–8)
Abnormal auditory evoked potentials	1 (5.6 (1.4–27))
Abnormal visual evoked potentials	1 (5.6 (1.4–27))
Parental concern about child development	3 (16.7 (3.5–41))
Extra tutoring during school	5 (27.8 (9.7–54))
Attended/attending kindergarten	18 (100.0 (81–100))

Data are given as *n* (% (95% CI)), median (interquartile range), *n* (%) or *n*/*N* (% (95% CI)). *Data missing for one case. GA, gestational age.

(Table 4). The mean score was 120 ± 1.9 for cognitive ability, 135 ± 16.7 for language ability and 127 ± 16.9 for motor ability.

Among those children assessed using the WISC-V tool, the mean intelligence quotient (IQ) was 96.2 ± 13.3. Two

Table 3 Genetic characteristics of seven infants born at term with severely low birth weight and diagnosed postnatally with genetic syndrome

BW (g)	*GA (weeks)*	*Gene*	*Genomic variant (nucleotide)*	*ACMG variant classification*	*Zygosity*	*Associated condition (inheritance pattern)*	*Age at dx (months)*
2160	37	*PTPN11*	NM_002834.3: c.467A > G (p.Asp156Gly); c.794G > A (p.Arg265Gln)	Likely pathogenic	Heterozygous (*cis*)	Noonan syndrome (AD)	2
1860	37	*ERCC6*	NM_000124.3 c.1690G > T (p.Glu564*)	Pathogenic	Homozygous	Cockayne syndrome (AR)	3
2240	39	*ERCC6*	NM_000124.3 c.2286 + 1G > T	Pathogenic	Homozygous	Cerebro-oculo-facio-skeletal syndrome-1 (AR)	15
1440	38	*XRCC4*	NM_001318012.1 c.25delC: c.732dupT	Pathogenic	Compound heterozygous	Short stature, microcephaly and endocrine dysfunction (AR)	29
2140	39	PQBP1	NM_005710 c.451_454del (p.Arg151*)	Pathogenic	Hemizygous	Renpenning syndrome (XLR)	36
1450	37	*H19*	Hypomethylation	Pathogenic	Heterozygous	Silver–Russell syndrome	4
2030	39	—	Uniparental disomy of chromosome 15	Pathogenic	Uniparental disomy	Prader–Willi syndrome	6

ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; BW, birth weight; dx, diagnosis; GA, gestational age at birth; XLR, X-linked recessive.

Table 4 Neurodevelopmental outcome in seven infants born at term with severely low birth weight, according to Bayley Scales of Infant and Toddler Development, 3rd edition, standardized with normative mean of 80–120

GA at birth (weeks)	*BW (g)*	*Postnatal findings*	*Family history*	*Sex*	*Clinical ES*	*Age at evaluation (mo + d)*	*Cognitive scale*		*Language scale*		*Motor scale*	
							Final score (95% CI)	*Adjusted age (mo)**	*Final score (95% CI)*	*Adjusted age (mo)**	*Final score (95% CI)*	*Adjusted age (mo)**
40 + 1	2000	—	FGR (sister)	F	Inc	23 + 26	105 (97–113)	> 42	121 (112–127)	> 42	112 (103–119)	> 42
37 + 4	2000	—	—	F	Inc	28 + 8	120 (110–126)	34	150 (139–154)	> 42	132 (123–138)	33–39
37 + 0	1950	Seizures	—	F	Inc	36 + 1	145 (133–149)	> 42	150 (142–157)	> 42	148 (137–152)	> 42
37 + 5	1910	Learning disabilities at school	—	M	Inc	27 + 20	100 (92–108)	28	†	†	112 (103–119)	28–31
37 + 7	1990	—	—	F	Inc	37 + 26	100 (92–108)	36	109 (101–116)	35–42	115 (106–121)	40–42
37 + 0	2300	ASD	Brugada syndrome (mother)	M	Inc	42 + 1	130 (119–135)	> 42	141 (131–145)	> 42	115 (106–121)	> 42
38 + 5	2230	—	SGA (brother)	F	Inc	42 + 6	140 (135–146)	> 42	141 (133–148)	> 42	154 (148–172)	> 42

As all children scored > 80 (i.e. above cut-off for average neurodevelopment) in all domains, qualitative classification was not included in table. *Age adjusted by test. †Language skills could not be assessed as child did not speak Spanish or Catalan. ASD, autism spectrum disorder; BW, birth weight; d, days; ES, exome sequencing; F, female; FGR, fetal growth restriction; GA, gestational age; Inc, inconclusive; M, male; mo, months; SGA, small-for-gestational age.

children were classified as having a low IQ, of which one was borderline (80) and the other was extremely low (68), the latter exhibiting low scores in all domains (Table 5). Mean scores for the five scales were as follows: 91.4 ± 21.9 for verbal comprehension, 100.7 ± 12.5 for visual spatial, 103.6 ± 15.8 for fluid reasoning, 94.0 ± 17.3 for working memory and 88.5 ± 11.5 for processing speed. Six (33%) children had a low or extremely low score in at least one of the five domains assessed. The scales with the greatest deviation from the norm were verbal comprehension and working memory, with three children attaining low or extremely low scores on each.

DISCUSSION

Main findings

This study demonstrates that among 63 infants from singleton pregnancies born severely small at term, with no prenatal evidence of placental insufficiency or structural anomaly, a postnatal genetic diagnosis was reported in

Table 5 Neurodevelopmental outcome in 11 infants born at term with severely low birth weight, according to Wechsler Intelligence Scale for Children, 5th edition

GA at birth (weeks)	*BW (g)*	*Postnatal findings*	*Family history*	*Sex*	*Clinical ES*	*Age at evaluation (years + months)*	*Total score*			*Verbal comprehension*			*Visual spatial*			*Fluid reasoning*			*Working memory*			*Processing speed*		
							FS	*P*	*QS*	*FS*	*P*	*QS*	*FS*	*P*	*QS*	*FS*	*P*	*QS*	*FS*	*P*	*QS*	*FS*	*P*	*QS*
37 + 5	2070	—	FGR (brother)	F	Inc	6 + 0	94	34	Avg	59	0.3	Ex low	111	77	Avg	112	79	Avg	122	93	Sup	105	63	Avg
39 + 0	1720	ASD	Severe VM, ACC (sister)	M	Inc	6 + 2	106	66	Avg	124	95	Sup	100	50	Avg	118	88	Avg–high	91	27	Avg	86	18	Low–avg
38 + 1	1990	—	ASD (sister), seizures (mother)	F	Inc	6 + 3	80	9	Low–avg	78	7	Low	105	63	Avg	94	34	Avg	88	21	Low–avg	69	2	Ex low
38 + 0	2086	—	FGR (brother)	F	Inc	6 + 6	93	32	Avg	106	66	Avg	92	30	Avg	85	16	Low–avg	85	16	Low–avg	83	13	Low–avg
38 + 8	2364	—	FGR (brother)	M	Inc	6 + 7	93	32	Avg	84	14	Low–avg	78	7	Low	88	21	Low–avg	82	12	Low–avg	92	30	Avg
40 + 0	2360	—	—	F	Inc	7 + 2	104	56	Avg	89	23	Low–avg	102	55	Avg	126	96	Sup	79	8	Low	75	5	Low
38 + 4	2140	—	—	F	Inc	7 + 4	100	50	Avg	81	10	Low–avg	105	63	Avg	103	58	Avg	112	79	Avg	108	70	Avg
36 + 5	1785	ASD, talipes	ASD (brother)	M	Inc	7 + 4	100	50	Avg	106	66	Avg	97	42	Avg	112	79	Avg–high	79	8	Low	86	18	Low–avg
37 + 0	1900	Seizures	SGA (brother)	F	Inc	8 + 0	118	88	Avg–high	124	95	Sup	117	87	Avg–high	117	87	Avg–high	117	87	Avg–high	89	23	Low–avg
38 + 0	2040	—	—	F	Inc	8 + 2	68	2	Ex low	62	1	Ex low	84	14	Low–avg	76	5	Low	72	3	Low	86	18	Low–avg
38 + 0	1800	—	CAH (mother)	F	Inc	8 + 6	102	55	Avg	92	30	Avg	117	87	Avg–high	109	73	Avg	107	68	Avg	95	37	Avg

Cut-offs were defined as follows: < 70, extremely low; 70–79, low; 80–89, low–average; 90–109, average; 110–119, average–high; 120–129, superior; ≥ 130, very superior. ACC, agenesis of the corpus callosum; ASD, autism spectrum disorder; Avg, average; BW, birth weight; CAH, congenital adrenal hyperplasia; ES, exome sequencing; Ex, extremely; F, female; FGR, fetal growth restriction; FS, final score; GA, gestational age; Inc, inconclusive; M, male; P, percentile; QS, qualitative score; SGA, small-for-gestational age; Sup, superior; VM, ventriculomegaly.

seven (11%), and six (33%) of the 18 non-syndromic infants that underwent developmental testing showed poor neurodevelopment.

Comparison with existing literature

Only two of the seven monogenic syndromes found in the present study were included in a recent review by our group of 20 monogenic syndromes typically associated with FGR[4], namely Noonan syndrome and Silver–Russell syndrome. Almost all postnatal short stature multigene panels include the *XRCC4* gene, a few contain the *ERCC6* gene, but none contains *PQBP1*. Two previous series have applied exome sequencing to non-malformed FGR babies in which FGR was defined either as a 'growth anomaly'[18] or 'estimated fetal weight < 10th centile'[19], including cases with abnormal Doppler and oligohydramnios. Overall, in the 55 fetuses evaluated, the diagnostic yield of exome sequencing was 13% (7/55). With very strict criteria to avoid including cases with placental insufficiency, our findings are consistent with the previous evidence, reporting an 11% incremental yield of exome sequencing over CMA.

Neurodevelopmental outcome

In term infants, neurodevelopmental outcome can be assessed without the confounding effect of prematurity. However, the wide heterogeneity in neurodevelopmental outcome reported in the literature could be attributed to differing definitions of SGA and the inclusion, without stratification, of cases with and without placental insufficiency, and those with and without a known genetic disease[20,21]. A population-based cohort study of 1 088 980 infants adjusted for maternal and paternal educational levels found that term SGA was associated significantly with an increased risk of poor school performance at the time of graduation from compulsory schooling[21]. However, this large cohort was 'contaminated' by a fraction of children with signs of placental insufficiency and genetic disease, which hinders the translation of these findings into real-setting counseling when, according to management guidelines, significant placental disease has been ruled out and a normal karyotype/CMA has been confirmed antenatally.

Our finding of a high prevalence of abnormal neurodevelopmental outcome is not likely to be secondary to latent undernourishment or perinatal hypoxia, since perinatal outcome was normal in all cases (reflected by umbilical artery pH at delivery and Apgar score) and because beyond a certain degree of placental insufficiency brain redistribution is present, which can be captured reliably on Doppler evaluation. One could argue that non-genetic syndromes (including endocrine disorders such as growth hormone congenital deficiency) or genetic syndromes not detectable by exome sequencing or CNV analysis may be operating. The fact that low scores were recorded only using the WISC-V and not the BSID-III tool could be explained by a delayed effect of being born small or by the superior accuracy of the WISC-V compared with the BSID-III; neurocognitive assessment in older infants is more accurate than in younger ones, since more relevant aspects of cognition can be evaluated.

Clinical implications

Counseling of parents with a severely SGA fetus once placental insufficiency and abnormal karyotype have been excluded is a clinical challenge. CMA offers an incremental improvement as it has a 5–10% greater diagnostic yield over conventional karyotyping in SGA fetuses[3]. However, the presence of a monogenic syndrome cannot easily be ruled out, since prenatal genetic findings only occasionally allow the suspicion of a syndrome with a causative gene. We found that in one of every nine fetuses in this clinical scenario, a genetic syndrome was diagnosed postnatally during follow-up. If exome sequencing had been considered prenatally, a postnatal diagnostic odyssey that in our series lasted for up to 3 years could have been avoided.

An additional source of complexity in prenatal counseling is our observation that among infants with normal clinical exome sequencing, the prevalence of abnormal neurodevelopment was 33%. Notably, one of our studied children had low scores for both working memory and processing speed, which may be indicative of ADHD[22]. Given that, in our series, exome sequencing was normal in those infants without a known syndrome, one may argue that the postnatal phenotypic expression of genetic syndromes associated with severe smallness is severe enough to allow efficient postnatal diagnosis.

Limitations and strengths

The main limitation of our work was the low (40%) uptake by eligible couples to participate in the study, which may be explained by the necessity for both parents and the child to be present during a 2-h intervention on a non-working day at the Hospital Clinic Barcelona during the Covid-19 pandemic. Selection bias and overestimation of the prevalence of adverse neurodevelopmental outcome may have occurred if parents with phenotypically abnormal children or those with evident developmental delay were more likely to accept the invitation to visit our hospital during pandemic times than were those with normal children. A third limitation of our study was the wide age range of participating children at the point of neurological assessment, together with the exclusion of children aged between 3.5 and 6 years at the time of execution of the study owing to the lack of appropriate assessment tools. Including infants in this age bracket may have provided greater insight into the neurodevelopment of the target population.

The main strength of our work was the originality of our approach, as no other series of severely small term infants without evidence of placental dysfunction has been reported, to the best of our knowledge. In addition, the fact that term delivery was an inclusion criterion avoids

any confusion arising from an adverse postnatal outcome related to iatrogenic prematurity rather than from the condition of FGR itself.

Conclusions

When counseling parents with a severely growth-restricted fetus at term, the 11% prevalence of genetic syndromes (beyond that detected by CMA) and 33% prevalence of poor neurodevelopmental outcome despite a normal genetic profile reported in the present study should be considered, but caution should be exercised, as larger prospective studies are warranted to confirm these preliminary findings.

ACKNOWLEDGMENT

This study was supported in part by a grant from Fondo de Investigaciones Sanitarias (grant #PI17/01153), Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional (FEDER), Ministerio de Economia y Competitividad, Spain.

REFERENCES

1. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, Silver RM, Wynia K, Ganzevoort W. Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound Obstet Gynecol* 2016; **48**: 333–339.
2. Lees C, Stampalija T, Hecher K. Diagnosis and management of fetal growth restriction: the ISUOG guideline and comparison with the SMFM guideline. *Ultrasound Obstet Gynecol* 2021; **57**: 884–887.
3. Borrell A, Grande M, Pauta M, Rodriguez-Revenga L, Figueras F. Chromosomal microarray analysis in fetuses with growth restriction and normal karyotype: a systematic review and meta-analysis. *Fetal Diagn Ther* 2018; **44**: 1–9.
4. Meler E, Sisterna S, Borrell A. Genetic syndromes associated with isolated fetal growth restriction. *Prenat Diagn* 2020; **40**: 432–446.
5. CME Review Article. *Pediatr Emerg Care* 2017; **33**: 792–793.
6. Mcintyre S, Blair E, Badawi N, Keogh J, Nelson KB. Antecedents of cerebral palsy and perinatal death in term and late preterm singletons. *Obstet Gynecol* 2013; **122**: 869–877.
7. Leonard H, Nassar N, Bourke J, Blair E, Mulroy S, De Klerk N, Bower C. Relation between intrauterine growth and subsequent intellectual disability in a ten-year population cohort of children in Western Australia. *Am J Epidemiol* 2008; **167**: 103–111.
8. Sucksdorff M, Lehtonen L, Chudal R, Suominen A, Joelsson P, Gissler M, Sourander A. Preterm birth and poor fetal growth as risk factors of attention-deficit/hyperactivity disorder. *Pediatrics* 2015; **136**: e599–608.
9. Lampi KM, Lehtonen L, Tran PL, Suominen A, Lehti V, Banerjee PN, Gissler M, Brown AS, Sourander A. Risk of autism spectrum disorders in low birth weight and small for gestational age infants. *J Pediatr* 2012; **161**: 830–836.
10. Arcangeli T, Thilaganathan B, Hooper R, Khan KS, Bhide A. Neurodevelopmental delay in small babies at term: a systematic review. *Ultrasound Obstet Gynecol* 2012; **40**: 267–275.
11. Figueras F, Gratacos E. An integrated approach to fetal growth restriction. *Best Pract Res Clin Obstet Gynaecol* 2017; **38**: 48–58.
12. Pauta M, Campos B, Segura-Puimedon M, Arca G, Nadal A, Tubau A, Perez SP, Marimon E, Martín L, López-Quesada E, Sabrià J, Muñoz B, Garcia E, Paz Y. Miño F, Borobio V, Gomez O, Eixarch E, Lopez M, Comas Rovira M, Borrell A. Next-generation sequencing gene panels and "solo" clinical exome sequencing applied in structurally abnormal fetuses. *Fetal Diagn Ther* 2021; **48**: 746–756.
13. Balasundaram P, Avulakunta ID. Bayley Scales Of Infant and Toddler Development. *StatPearls*. November 2021. https://www.ncbi.nlm.nih.gov/books/NBK567715/. [Accessed July 22, 2022].
14. Madaschi V, Mecca TP, Macedo EC, Paula CS. Bayley-III scales of infant and toddler development: Transcultural adaptation and psychometric properties. *Paidéia* 2016; **26**: 189–197.
15. Fenollar-Cortés J, Watkins MW. Construct validity of the Spanish Version of the Wechsler Intelligence Scale for Children Fifth Edition (WISC-V Spain). *Int J School Educ Psychol* 2018; **7**: 150–164.
16. Wechsler D. *Escala de inteligencia de Wechsler para niños, quinta edición: WISC-V (edición original, 2014)*. Pearson: Madrid, Spain, 2015.
17. Johnson S, Moore T, Marlow N. Using the Bayley-III to assess neurodevelopmental delay: which cut-off should be used? *Pediatr Res* 2014; **75**: 670–674.
18. Zhou J, Yang Z, Sun J, Liu L, Zhou X, Liu F, Xing Y, Cui S, Xiong S, Liu X, Yang Y, Wei X, Zou G, Wang Z, Wei X, Wang Y, Zhang Y, Yan S, Wu F, Zeng F, Wang J, Duan T, Peng Z, Sun L. Whole genome sequencing in the evaluation of fetal structural anomalies: a parallel test with chromosomal microarray plus whole exome sequencing. *Genes (Basel)* 2021; **12**: 376.
19. Gabriel H, Korinth D, Ritthaler M, Schulte B, Battke F, von Kaisenberg C, Wüstemann M, Schulze B, Friedrich-Freksa A, Pfeiffer L, Entezami M, Schröer A, Bürger J, Schwaibold EMC, Lebek H, Biskup S. Trio exome sequencing is highly relevant in prenatal diagnostics. *Prenat Diagn* 2022; **42**: 845–851.
20. Vollmer B, Edmonds CJ. School age neurological and cognitive outcomes of fetal growth retardation or small for gestational age birth weight. *Front Endocrinol (Lausanne)* 2019; **10**: 186.
21. Lindström L, Wikström AK, Bergman E, Lundgren M. Born small for gestational age and poor school performance – how small is too small? *Horm Res Paediatr* 2017; **88**: 215–223.
22. Takayanagi M, Kawasaki Y, Shinomiya M, Hiroshi H, Okada S, Ino T, Sakai K, Murakami K, Ishida R, Mizuno K, Niwa SI. Review of cognitive characteristics of autism spectrum disorder using performance on six subtests on four versions of the Wechsler Intelligence Scale for Children. *J Autism Dev Disord* 2022; **52**: 240–253.