Supplemental Methods

Dried Blood Spot Quality Control Material

Dried blood spot (DBS) quality control material was created for use as positive and negative controls for all qPCR and MLPA assays. Immortalized B-cell lines from confirmed patients with the 22q11.2 deletion, GM07939, GM17938 and GM17942 (Coriell, New Jersey, USA), were washed twice in 1X PBS (Invitrogen, California, USA), before being mixed with saline-washed red blood cells (RBC) devoid of lymphocytes and mixed to a 50% hematocrit level with fetal bovine serum (Sigma, Missouri, USA). The mixture was then allowed to mix for > 1 hour on a Nutator (VWR, Pennsylvania, USA). Both the B-cell line-RBC mixture and whole blood from a wild-type (no 22q11.2 deletion) donor were applied to Whatman 903 Protein Saver cards (VWR) using a pipette (75 µl) and allowed to dry overnight at room temperature.

DNA Extraction from DBS for qPCR

A single 3.2 mm disc was punched from each de-identified NBS DBS using the BSD 600 Plus (Luminex, Texas, USA) into a 96-well-U-bottom polypropylene microtiter plate (Corning, New York, USA). DNA was extracted on an NXp Span-8 liquid handler (Beckman Coulter, California, USA) using the following method. Each DBS was washed twice with 110 µl of 20 mM Tris-HCl pH 9.0, 0.5% Triton-X, and once with 110 µl of 20 mM Tris-HCl pH 9.0, each wash consisting of a ten-minute incubation at 37°C and 700 rpm on a heated microplate shaker. Then, 50 µl of Tris-HCl pH 9.0, 50 ng/µl yeast tRNA was added and plates were sealed with pierceable aluminum heat seal and sealer (Axygen, New York, USA) to prevent contamination and evaporation. DNA was eluted by incubation at 98°C for 40 minutes with shaking in a VorTemp 56 (Labnet, New Jersey, USA). DNA plates were then stored at -20°C until completely frozen. DBS quality control material consisting of 22q11.2 deletion-positive and wild-type (i.e.,

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22q11.2 deletion negative) in-house made samples (as above) were punched and extracted along with population-based NBS samples on each 96-well plate for use in quantifying gene copy number, thus appearing four times each on a 384-well initial qPCR screening assay, and in duplicate on the secondary qPCR assay.

Quantitative PCR Assays

Relative quantification using a quadplexed 5'-hydrolysis qPCR initial assay was carried out in a 20 µl reaction volume on a 384-well plate (Life Technologies, California, USA). Each reaction contained 1X DurAmp Mastermix (Life Technologies), 400 nM for each gene specific primer (IDT, Iowa, USA), 200 nM for each gene specific probe (LGC BioSearch Technologies, Teddington, UK), i.e., for three 22q11.2 deletion region genes (Figure 1), *UFD1L, COMT, CRKL*, and a standard reference gene *RPPH1* (encoding RNase P), together with 8 µl of extracted DNA. The primers and probes for the primary screening assay are listed in Tables S1 and S2. On the initial qPCR assay, a screen-positive sample for the 22q11.2 deletion was defined as qPCR results consistent with the apparent deletion of all three 22q11.2 deletion region probes, or the apparent deletion of both *UFD1L* and *COMT* probes. This represented a refining of methodology for these three probes originally tested using spiked samples from individuals known to have 22q11.2 deletions (Wisconsin, unpublished data).

Screen-positive samples that had sufficient DNA for a second DBS punch were subjected to a secondary multiplexed qPCR assay run in a 20 µl total volume in a 96-well FAST qPCR plate (Life Technologies) that consisted of the following two target genes: *TBX1* (22q11.2 deletion region, Figure 1), and *RPPH1* (reference). Each reaction contained 1X DurAmp Mastermix (Life Technologies), 900 nM for the *TBX1* primers, 250 nM for the *TBX1* probe[1] (Life Technologies, Cat#Hs01313390_cn FAM), 400 nM for the *RPPH1* primers, and 200 nM

for the *RPPH1* probe. The primer and probe sequences for *RPPH1* are listed in Tables S1 and S2. The primer and probe sequences for *TBX1* are not listed as they are proprietary.

Primer and probe sequences were assessed against the human genome using the BLAST program to ensure 100% homology to only the sequence from which they were derived. The DurAmp master mix contains the ROX dye as an internal passive reference to which the reporter dye signal can be normalized by the software.

qPCR for both assays was performed using the Comparative Ct (ΔΔCt) mode on the Applied Biosystems ViiA 7 Real-Time PCR System using the following PCR cycling parameters: 1 cycle at 95°C (45 seconds) followed by 45 cycles at 95°C (30 seconds) and 60°C (1 minute 30 seconds).

qPCR Data Analysis

Relative quantification (RQ) of each gene was calculated using the ViiA 7 software v.1.2 (Life Technologies) by way of presenting the ratio of the 22q11.2 genes of interest, relative to the *RPPH1* reference gene from the de-identified NBS samples, compared to that of our wild-type quality control samples, following the ΔΔCT method.[2] The area under the Receiver Operating Characteristic (ROC) curve was calculated using non-parametric methods[3] to determine assay cut-off. The RQ cut-off values for a heterozygous 22q11.2 deletion are presented in Table S3.

Salting-Out Extraction of DNA from DBS for MLPA

Additional DBS punches were required for the confirmatory MLPA assay that used standard probes from the 22q11.2 region. A single 3.2 mm disc was punched (Wallac DBS Puncher) and transferred to a 1.5 mL microtube followed by overnight lysis with 150 µl of STE buffer (1M Tris-HCl pH 8.5, 3M NaCl, 0.5M EDTA) plus 50 µl of proteinase K (20mg/mL;

Sigma) at 56°C. The next day, 200 µl of lysis buffer (Nuclisens) was added and incubated at 56°C for 2 hours. Then, 250 µl of 7.5M NH_4OAc was added and the tube placed at -20°C for 2.5 hours, followed by centrifugation at 14.5k rpm for 15 minutes at room temperature. The supernatant (620 µl) was transferred to a fresh microtube and the following was added: 1 µl of glycogen (20mg/ml, Roche, Basel, Switzerland), 68.9 µl of 3M NaOAc, and 482.2 µl of isopropanol. The tube was mixed well and centrifuged at 14.5k rpm for 20 minutes at room temperature. The DNA pellet was washed with 1 mL of room temperature 70% ethanol and centrifuged at 14.5k rpm for 20 minutes at room temperature. The DNA pellet was left to air dry for up to 20 minutes and then resuspended in 10 µl of 10mM Tris pH 8.0 and heated at 55°C for 1-2 hours with gentle shaking every 20 minutes.

MLPA Confirmatory Assay

Screen-positive 22q11.2 deletion samples, in addition to six screen-negative samples used as controls for normalization, were assayed by MLPA with the SALSA® MLPA® Probemix P250-B2 "DiGeorge" MLPA kit according to the general protocol provided by the manufacturer (MRC Holland, Amsterdam, Netherlands). We used a maximum of 100 ng of DNA extracted from DBS. MLPA was performed on an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher, Massachusetts, USA) and analysis was carried out with Coffalyser.Net software (MRC Holland). The dosage quotient (DQ) for heterozygous deletions was defined as $0.40 < DQ < 0.65$, as per MRC Holland guidelines for the kit.

Sample Size and Clinical Variables

For sample size selection, power analyses were conducted. For a sample size of 30,000 and an alpha of 0.05, the power required to detect a 22q11.2 deletion prevalence of 1 in 2000 is

0.78. For the same sized sample, this power decreases to 0.56 with a 22q11.2 deletion prevalence of 1 in 3000, and further decreases to 0.49 with a prevalence of 1 in 4000.

For the subset of NBS samples from singletons born at term with sex, birth weight, and gestational age data available (n=26,318) we investigated the following clinical variables: sex, birth weight for gestational age, the proportion of newborns transfused at birth, the proportion of newborns with a complex neonatal feeding type, gestational age, and maternal age at birth. A minority of samples had missing data for any one of these variables, thus we have listed the sample sizes for each in the footnotes of Table 1. Main comparisons were between samples with a confirmed 22q11.2 deletion and the remaining population-based samples. We calculated the proportions of each group considered <3rd and <10th percentile for sex- and gestational age-corrected birth weights, based on a Canadian reference sample.[4] For the data on feeding available, those designated as "total parenteral nutrition (TPN)" or "nil per os" as the sole or one of several feeding types (e.g., breast and TPN), were classified as having a complex neonatal feeding type.

Supplemental Results

A total of 63 samples had screen-positive initial qPCR assay results. MLPA results confirmed a pathogenic 22q11.2 deletion in fourteen samples (Figure S1), and confirmed the absence of the 22q11.2 deletion in the remaining 49 samples, with one of these 49 samples showing a smaller nested 22q11.2 deletion (Figure S2D). MLPA results for the proximal nested LCR22A-LCR22B and LCR22A-LCR22C 22q11.2 deletions, the common LCR22A-LCR22D 22q11.2 deletion, and a small nested 22q11.2 deletion that did not meet study criteria are shown in Figure S2.

Supplemental Tables

Table S1. Primers used in the primary qPCR screening assay for 22q11.2 deletions

Primers	**Sequence 5' → 3'**
UFD1L FP	GTTTGACTTGGAAGTGGAGCAGCAG
UFD1L RP	GGAGCTCCCCTCAAGCTGAAAG
COMT FP	CAGTTGTGGTTACTTTCTGGAGAGAG
COMT RP	GGCCGCCCAGGAAGAC
CRKL FP	GAGAGAAGCCTGAAGAACAGTGG
CRKL RP	CTTTTCGACATAAGGGACAGGAAT
RPPH1 FP	TTGCCGGAGCTTGGAACAG
RPPH1 RP	ACCTCACCTCAGCCATTGAAC

FP: forward primer. RP: reverse primer.

UFD1L, COMT, and *CRKL* are from the 22q11.2 deletion region; *RPPH1* is a standard reference used for qPCR.

Table S2. Probes used in the primary qPCR screening assay for 22q11.2 deletions

Probe	**Sequence 5' → 3'**
UFD1L	AAGACAAAGAGCTGTCCCTGAGGG-FAM
COMT	CCTCCAGCTCCTGCAT-Quasar 670
CRKL	TGCCCGGAACAAGGATGGCC-Quasar 705
RPPH1	CTCACGGCCAGCGAAGT-VIC

Table S3: qPCR RQ cut-off values for 22q11.2 deletion region gene targets indicating a possible 22q11.2 deletion (i.e., screen-positive)

Probe	Target cut-off (i.e., RQ less than:)
Primary qPCR screening assay	
UFD1L	0.713
COMT	0.692
CRKL	0.776
Secondary qPCR screening assay	
TBX1	0.620

RQ = relative quantification.

See Supplemental Methods above for details of assays.

Note: The *TBX1* qPCR screening assay showed mixed results. Of 12 MLPA-confirmed 22q11.2 deletion samples with *TBX1* results, nine (75%) indicated a 22q11.2 deletion (RQ range 0.530-0.607), and three had results above the cut-off (RQ 0.621, 0.650 and 0.657). For the remaining 49 primary screen-positive samples, *TBX1* results were available for 44, 43 (97.7%) of which indicated no 22q11.2 deletion (RQ range 0.672-1.314) and one confirmed to have no 22q11.2 deletion on MLPA despite a *TBX1* result (RQ 0.614) below the cut-off.

Supplemental Figures

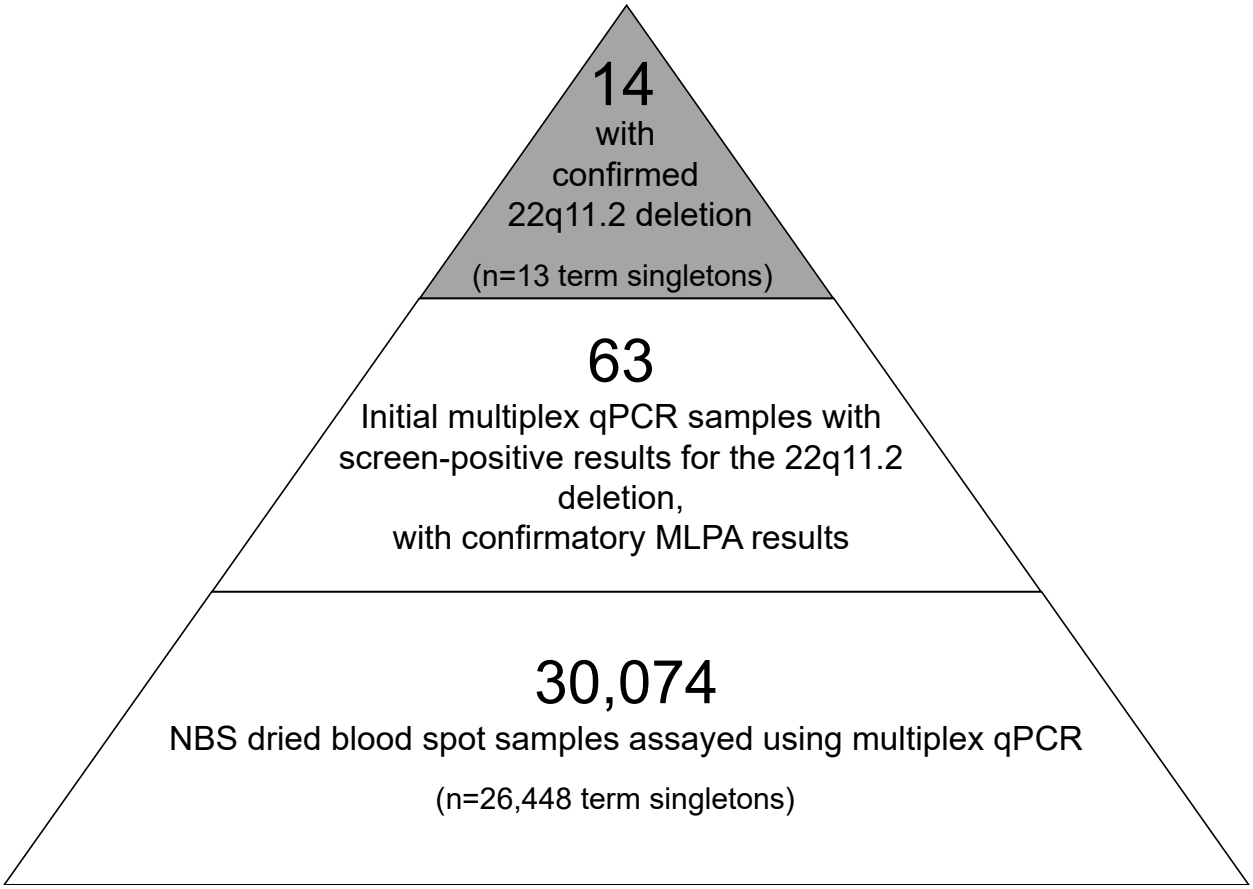

Figure S1. Sample and summary of screening and confirmatory results leading to a minimum live birth prevalence estimate for 22q11.2 deletions. MLPA results were available for all samples that were screen-positive by the primary qPCR assay (n=63). 14 were confirmed to have a 22q11.2 deletion (11 with a common LCR22A-LCR22D deletion, 3 with a proximal nested deletion, see Figure 1); 49 did not meet our pre-established criteria for a pathogenic 22q11.2 deletion, 48 with MLPA-confirmed absence of the deletion and one with a small nested proximal 22q11.2 deletion (Figure S2D).

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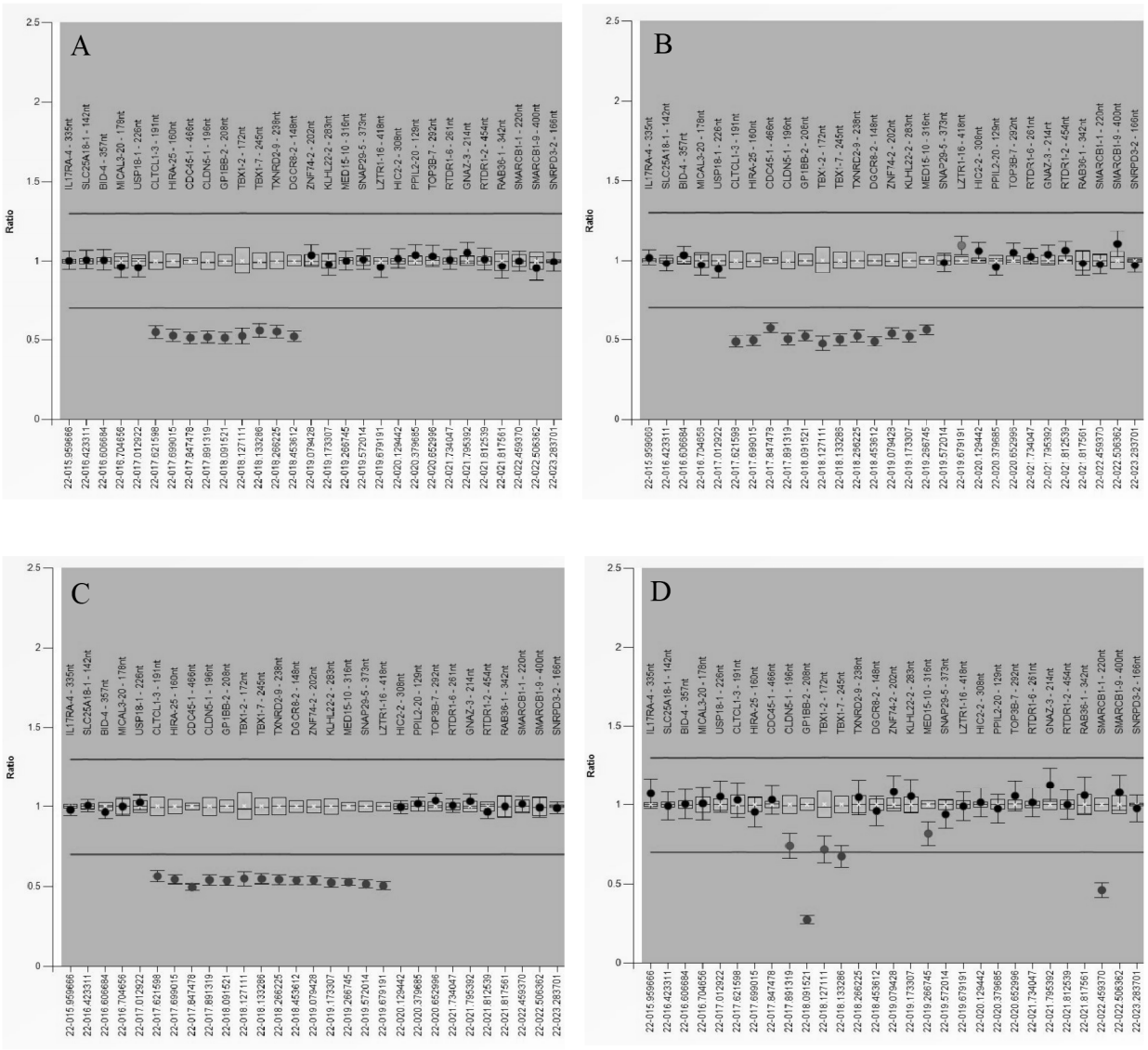

Figure S2. MLPA results for 22q11.2 deletions. The MLPA probes (MRC Holland) are listed at the top of the figure, from centromeric (left) to telomeric (right); for relative position of probes see Figure 1. The circles with error bars indicate the dosage quotient for each probe. The bottom horizontal line represents the cut-off point provided for a heterozygous 22q11.2 deletion.

A. Proximal nested LCR22A-LCR22B 22q11.2 deletion.

B. Proximal nested LCR22A-LCR22C 22q11.2 deletion.

C. Common LCR22A-LCR22D 22q11.2 deletion.

D. Small nested (atypical) 22q11.2 deletion (not meeting study criteria).

Supplemental References

1. Tomita-Mitchell, A. *et al.* Multiplexed quantitative real-time PCR to detect 22q11.2 deletion in patients with congenital heart disease. *Physiol. Genomics* **42A**, 52–60 (2010).
2. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods San Diego Calif* **25**, 402–408 (2001).
3. DeLong, E. R., DeLong, D. M. & Clarke-Pearson, D. L. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* **44**, 837–845 (1988).
4. Kramer, M. S. *et al.* A new and improved population-based Canadian reference for birth weight for gestational age. *Pediatrics* **108**, E35 (2001).