Systematic assessment of atypical deletions reveals genotype-phenotype correlation in 22q11.2

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Running title: Atypical 22q11.2 deletions

Keywords: 22q11.2 atypical deletions, affected sib pair, congenital heart defects, mental retardation, choanal atresia

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Abstract

Introduction
Clinical variability associated with the common 22q11.2 microdeletion is well known and led to broad application of FISH diagnostics with probes for loci TUPLE1 or D22S75 (N25), although rarely reported atypical deletions associated with the same phenotypic spectrum would not be discovered by these probes. As most types of 22q11.2 deletions occurred between low copy repeats within the region (LCR22), we assumed that atypical deletions would be more common than reported. To address this question and the possibility of a deletion size related genotype-phenotype correlation, we systematically assessed the frequency of typical and atypical 22q11.2 deletions in a large cohort of patients.

Methods
We used a set of 10 FISH DNA probes which is capable to detect all reported and hypothetical types of deletions in between the LCR22, and analysed a total of 350 patients. Deletion sizes in atypical deletions were established by use of further FISH probes. Frequency of certain atypical deletions was analysed in controls by FISH and quantitative PCR.

Results
Patients with conotruncal heart defects (ctCHD) and with typical VCFS phenotype showed the common 3 Mb or nested 1.5 Mb deletions in 18.5 % and 78.6 %, respectively, but no atypical deletion, while 5 % (3/63) of patients with mildly suggestive, atypical phenotype showed atypical distal deletions, which were neither detected in patients with mental retardation of unknown origin nor in healthy controls.

Discussion
These statistically significant differences demonstrate that atypical distal 22q11.2 deletions are very uncommon in patients with ctCHDs, while atypical congenital heart defects and mild dysmorphism is a recognizable feature of atypical distal deletions. Further phenotype-genotype analysis disclosed association of significant developmental delay with the distal part of the common deletion region and choanal atresia and atypical CHDs with the adjacent distal deletion region.

Keywords: 22q11.2 atypical deletions, affected sib pair, congenital heart defects, mental retardation, choanal atresia
Introduction
The well known recurrent ~3 Mb microdeletion within chromosomal band 22q11.2 is associated with a variety of symptoms and syndromes \(^1\), including DiGeorge and velocardiofacial syndromes (DGS/VCFS; MIM 188400), and constitutes a major cause of congenital heart disease (CHD), accounting for about 5 % of all CHDs in live born \(^4\). Despite variable phenotypes even within families \(^3\), \(^5\), 84-90 % of N25/TUPLE1 deleted VCFS patients show an identical deletion size of approximately 3 Mb, while 7-14 % were shown to have a proximally nested ~1.5 Mb deletion \(^6\),\(^7\), \(^8\). In contrast, Kurahashi et al. \(^9\) investigated 100 patients from the outset with several FISH probes from the 3 Mb region and in addition detected a small distally nested deletion in one patient. A few patients with typical phenotype but normal results for the commonly used probes were further investigated and reported to have atypical nested \(^10\),\(^14\), but also adjacent atypical distal deletions \(^15\),\(^16\). As both, common and atypical deletions were shown to be mediated by several low copy repeats (LCR) within the region \(^6\), \(^15\), \(^17\), \(^18\), we suspected that atypical deletions would be more common than reported if investigated systematically. We therefore analysed 350 patients with symptoms of the DGS/VCFS spectrum with a set of 10 FISH probes which would detect all reported and hypothetical types of deletions in between the LCRs.

Subjects and Methods

Patients
We initially analysed 73 patients with conotruncal heart defects (ctCHD) such as interrupted aortic arch, truncus arteriosus communis, Fallot tetralogy or pulmonary atresia, recruited from the pediatric cardiology centres of the Universities of Erlangen and Tuebingen, which formerly showed normal results for the FISH probe N25 (D22S75). In addition we analysed prospectively 200 patients with conotruncal heart defects. Clinical details of these patients have been published elsewhere or are in preparation \(^19\)-\(^23\). While the latter patients were recruited only for the characteristic heart phenotype, 77 further patients missing characteristic ctCHDs were analysed because clinical investigation at the genetic clinic due to developmental delay and/or malformations (MR/MCA) of unknown origin revealed different features consistent with the 22q11.2 deletion spectrum. Inclusion criteria for the latter were either developmental delay, short stature or frequent infections in addition to at least two minor facial anomalies compatible with the DGS/VCFS spectrum, or presence of at least one minor dysmorphism in addition to one of the following anomalies: congenital heart defect, urogenital anomaly, cleft palate, choanal atresia, hypocalcemia. Only 14 of these latter patients were clinically judged as having a typical DGS/VCFS facial phenotype. In addition one affected sib pair from healthy parents known from the German parents support group was analysed for deletion size and parental origin. As control groups up to 300 patients with MR/MCA of unknown origin not meeting the inclusion criteria for the 22q11.2 deletion study and 285 health controls were analysed for certain deletions. The study was approved by the University ethical review board and appropriate informed consent was obtained from human subjects.

Methods
FISH analysis was performed on metaphase spreads from cultivated lymphocytes with following 10 DNA probes covering the common and atypical deletion intervals: 6E8 (D22S427), 109G12 (109G12-1) \(^17\), 51H3 (D22S1649), 70A2 (D22S1694), Pac 140D4 (TUPLE1/HIRA), D0832 (COMT) \(^8\), 48c12 (D22S264) \(^18\), co23 (UFD1L) \(^24\), cHKAD26 (D22S935) \(^25\), BAC 438P22 (D22S425) \(^15\). Probes were directly labelled with Cy3 by nick translation with a Roche corp. kit and hybridised with a 22q subtelomeric control probe (GS-98-C4, locus 22QTEL31) directly labelled with FluoroX as described earlier \(^26\). In patients with atypical deletions and their parents according microsatellite markers and either probes RP11-140M6 and RP11-134C5 or a commercial bcr/able probe (ONCOR), RP11-1146O18
and RP11-20P18 were analysed as described 27. MR/MCA controls were analysed by FISH with the probes Pac140D4, cHKAD26 and BAC438P22. Healthy controls were analysed by quantitative PCR using Taqman™ assays with minor groove binding probes derived from exon 2 of CRKL and of exon 1 of GNAZ. Primer and probe sequences used were as follows: CRKLe2F2-GTGGAGTGCCCGGAACAA, CRKLe2R2-CTTTTCGACATAAGGGACAGGAAT, CRKLe2_mgb_probe2-ATGGCCGGGTTGGG, GNAZe1F-CGGGCATTGTGGAGAACAA, GNAZe1R-CCCAGCTCCACCACCTTGA, GNAZe1_mgb_probe-CTTCAAGGAGCTCACC. The assays were performed with threefold measurements each multiplexed with an albumin probe as an internal reference and calculation of copy numbers by the ∆∆Ct method as described elsewhere 28. For each assay two patients deleted for the respective region were co-analysed as positive controls.

Results
In the 73 retrospective patients with ctCHD with normal results for locus D22S75 no further deletion was detected. 37 of the 200 (18.5 %) prospective patients with ctCHD, 11 of the 14 (78.6 %) typical VCFS patients and 3 of the 63 (4.8 %) weakly suggestive of VCFS patients showed a deletion within 22q11.2. 44 of 51 deletions (86.3 %) had the common ~3 Mb size encompassing probes c51H3, c70A2, P140D4, co23, D0832, c48c12, and cHKAD26, and three (5.9 %) represented the proximally nested ~1.5 Mb deletion including probes c51H3, c70A2, P140D4, co23, and D0832, only (table 1). One proximal deletion in addition to the former probes was proximally expanded into the cat-eye syndrome region (43101, figure 1). Three deletions (5.9 %) were distal deletions, one including c48c12 and cHKAD26 (patient 77104), thus nested within the common 3 Mb region, overlapping with one reported deletion 11, and two were distally adjacent to the 3 Mb region, including B438P22 and BCR, only, in one patient (07604), and additionally 109G12 in another (31502), thus overlapping the deletion reported earlier by our group 15 (figure 1).

The common ~3 Mb deletion were detected in ctCHD patients and the patients clinically judged as typical for DGS/VCFS, only. The proximally nested and enlarged ~1.5 Mb deletions were seen in 3 patients with typical ctCHD and one patient with VCFS but normal development, while all three distal deletions were disclosed in patients only weakly suggestive of 22q11.2 deletion. The different frequencies of atypical deletions were statistically significant (table 2). Patient 77104 with the ~1 Mb nested distal deletion was referred for speech delay, but otherwise showed only mild hypotonia, frequent infections and attacks of pseudo croup as well as a small mouth, mild retrognathia and mild ptosis suggestive of DGS/VCFS. At the age of 6 years he was mildly mentally retarded and suffered from increased anxiety and gave the impression of psychological instability, but formal testing was pending (figure 2 c, d; table 3). The deletion was excluded in his mother, but his father, who was said to suffer from schizophrenia was not available for analysis. Patient 31502 with the ~0.6 Mb deletion in the BCR region was referred at the age of 8½ month because of her valvular pulmonic stenosis with muscular VSD, small PFO, and PDA. Her appearance and psychomotor development was normal, and only very careful investigation disclosed minimal broad folding of the right helix and somewhat widely spaced inverted nipples (figure 2 g-i). Her healthy father, who had narrow palpebral fissures and low set ears was shown to carry the same deletion (Figure 2 j, k). Further relatives of the father were not available for investigation. Patient 07604 with the second atypical distal deletion of about 2.1 Mb in the IGLV region was referred at the age of 7½ years with the diagnosis of CHARGE association due to bilateral choanal atresia, right sided preauricular tag, hypoplastic irides, small VSD, and conductive hearing loss (Figure 2 e, f). In addition she showed frequent infections, strabism, hyperopia, and learning difficulties with a low normal average IQ of 85 (Kaufmann-Assessment Battery for children). The deletion was excluded in both healthy parents.
The atypical distal deletions were neither detected in up to 300 patients with MR/MCA of unknown origin not fulfilling the inclusion criteria for the 22q11.2 study nor in 285 healthy controls.

Sizing of the 22q11.2 deletion in the affected sib pair, whose parents both had no deletion, unexpectedly showed a common 3 Mb deletion in the boy and a proximally nested 1.5 Mb deletion in the girl. From the 8 polymorphic markers out of the deleted regions tested, only D22S941 was fully informative, indicating a paternally derived deletion in the boy and a maternally derived deletion in the girl. D22S1623 was informative for the boy and confirmed a paternally derived deletion. To further confirm different parental origin of the deletions, 23 SNPs from the TBX1 genomic region, where both siblings are hemizygous were analysed and showed divergent alleles at 11 SNPs. While both had a mild expression of the typical DGS/VCFS facial appearance and frequent infections in infancy, the boy also had muscular hypotonia and mild mental retardation, while the girl attended regular school with only minor hyperactivity problems, most probably caused by prolonged neonatal meningitis (figure 2 a, b).

Table 1: Overview of Results in different patient groups

<table>
<thead>
<tr>
<th>N</th>
<th>Phenotype</th>
<th>Common 3 Mb deletion</th>
<th>Proximal 1.5 Mb deletion</th>
<th>Distally nested deletion</th>
<th>Adjacent distal deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>ctCHD without common deletion</td>
<td>--</td>
<td>--</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>ctCHD</td>
<td>33</td>
<td>3+1*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>VCFS</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>63</td>
<td>Atypical “VCFS”</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>MCA/MR</td>
<td>n. a.</td>
<td>n. a.</td>
<td>n. a.</td>
<td>n. a.</td>
</tr>
<tr>
<td>250</td>
<td>MCA/MR</td>
<td>n. a.</td>
<td>n. a.</td>
<td>0</td>
<td>n. a.</td>
</tr>
<tr>
<td>300</td>
<td>MCA/MR</td>
<td>n. a.</td>
<td>n. a.</td>
<td>n. a.</td>
<td>0</td>
</tr>
<tr>
<td>285</td>
<td>Healthy controls</td>
<td>n. a.</td>
<td>n. a.</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

ctCHD: conotruncal congenital heart defect; --: not applicable; n. a.: not analysed; *one deletion includes the cat-eye region, MCA/MR: multiple congenital anomalies/mental retardation; VCFS: velocardiofacial syndrome.

Table 2: Statistical evaluation of significance of different frequencies of atypical distal deletions by Fisher’s exact testing

<table>
<thead>
<tr>
<th>Compared Groups</th>
<th>Parameters</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ctCHD vs. atypical VCFS</td>
<td>0/273 vs. 3/63</td>
<td>p = 0.007</td>
</tr>
<tr>
<td>MCA/MR vs. atypical VCFS</td>
<td>0/250 vs. 3/63</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>Healthy controls vs. atypical VCFS</td>
<td>0/285 vs. 3/63</td>
<td>p = 0.005</td>
</tr>
</tbody>
</table>
Table 3: Summary of Phenotypic findings according to different atypical small 22q11.2 deletions

<table>
<thead>
<tr>
<th>Deleted Interval (see Fig. 1)</th>
<th>Common deletion*</th>
<th>I-III</th>
<th>II</th>
<th>IIa</th>
<th>IIb</th>
<th>IIIb</th>
<th>I+IV</th>
<th>V</th>
<th>IV+V</th>
<th>VI</th>
<th>IGLV</th>
<th>VII</th>
<th>V+V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>558</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Premature death</td>
<td>8 %</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>CHD</td>
<td>75 %</td>
<td>TOF</td>
<td>IAA B</td>
<td>TOF</td>
<td>-</td>
<td>VSD</td>
<td>PA/SD</td>
<td>-</td>
<td>TOF</td>
<td>TAC2</td>
<td>-</td>
<td>PSt</td>
<td>-</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>60 %</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Urogenital Anomalies</td>
<td>36 %</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VPI</td>
<td>32 %</td>
<td>+</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Cleft palate</td>
<td>14 %</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tracheo/laryngomalacia</td>
<td>3 %</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Choanal stenosis/atresia</td>
<td>1 %</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Iris coloboma</td>
<td>&lt; 1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Profound immunodeficiency</td>
<td>n. a.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Psychosis</td>
<td>18 %</td>
<td>n. a.</td>
<td>n. a.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>n. a.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>38 %</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>1</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Short stature</td>
<td>36 %</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Typical face</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>A</td>
<td>(A)</td>
<td>A</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* according to Ryan et al. 1997; VPI: velopharyngeal insufficiency; TOF= tetralogy of Fallot; IAA B: interrupted aortie arch type B; VSD: ventricular septal defect; TAC2/3: truncus arteriosus communis type 2 or 3; A: minor facial anomalies; PSt: pulmonal artery stenosis; n. a. not applicable due to severe hypoxic brain damage during heart surgery.

Discussion

To date even with reference to non-overlapping deletions, no consistent genotype-phenotype correlation evolved in 22q11.2 deletions. Nevertheless, our systematic assessment of typical and atypical 22q11.2 deletions in a large number of patients clearly demonstrates a significant correlation between deletion site and phenotypic expression. Although single case exceptions have been reported, our results revealed that if conotruncal heart defects or Shprintzen/VCFS syndromes are related to 22q11.2 deletion, they are usually associated with the common 3 Mb or the frequent 1.5 Mb proximally nested deletions, both encompassing the T-box transcription factor TBX1. This is in line with the finding of Yagi et al. who revealed a deletion with the proximal N25 probe in 96% of 235 typical DGS/conotruncal anomaly face syndrome patients, but no atypical deletion in the remaining 10 patients investigated with further probes. Moreover, in 3 of these remaining 10 patients one frameshift and two missense mutations within TBX1 were detected, assigning the main DGS/VCFS phenotype such as facial anomaly, conotruncal heart defects, hypoplastic thymus, parathyroid dysfunction, and velopharyngeal insufficiency to TBX1 haploinsufficiency. Even though the role of TBX1 in heart development was also demonstrated in mouse models, there is some evidence for additional heart genes within the region such as Crkl, Hira, and Ufd1l. Therefore it seems likely that additional genes contribute to CHD in patients with atypical deletions excluding TBX1, although positional effects might be possible.
Like the patients with TBX1 mutations, none of our four patients with the proximal 1.5 Mb nested deletion showed severe learning disability or mental retardation, which is found in 38% of 22q11.2 deletion patients 1. Accordingly, Bartsch et al. 34 reported four patients with assumed similar ~1.5 Mb deletions, which displayed typical DGS/VCFS organic problems but both older patients showing normal development. As individual mental development is subject to multifactorial influences including familial background, our finding of correlation of mental development with different deletion size within the same family provides further evidence for this suggestive correlation, as does our patient 77104 with the distally nested deletion who showed significant developmental delay and possibly early signs of psychiatric illness 35. Learning disabilities were also reported by Garcia et al. 11 in their case with similar distal deletion.

The unexpected finding of different deletion sizes and different parent of origin within our siblings from non-deleted parents further emphasizes the necessity of further investigations in affected siblings before counselling for germ line mosaicism. Accordingly, independent de novo 22q11.2 deletions were previously reported in first cousins 36.

The frequency of atypical distal deletions non-overlapping with the common deletion region in our patients with only mildly suggestive features of the DGS/VCFS spectrum differs significantly from the lack of these deletions in 285 healthy controls, up to 300 patients with unclassified mental retardation and in 273 patients with ctCHD. Accordingly, development was normal in the patient and father with the smaller deletion distally nested within the adjacent region (31502), and only mildly delayed in the present and published patients with the larger adjacent deletions (07604, 15), as well as in the smaller deletion proximally nested within the adjacent deletion region in a published patient 16. Although exceptions are reported, the type of CHD of our new patients with deletions distally to the common 3 Mb region is not characteristic for 22q11.2 deletion in the majority of cases 1, and their very mild minor anomalies would usually not be considered relevant. Interestingly, two of the 5 patients known with a deletion of the D22S308 region had choanal atresia versus only 1% of patients with common deletions 1. As choanal atresia is a major feature of CHARGE syndrome, some of the CHD7 mutation negative patients 37 might therefore have an atypical distal 22q11.2 deletion.

It is obvious, that phenotypic expression in 22q11.2 deletions is modified by factors others than deletion sizes, which hampers the identification of their contribution. However, our systematic approach reveals a not fully penetrant genotype-phenotype correlation with a critical role of the proximally nested TBX1 containing interval for ctCHD and the typical DGS/VCFS facial features, of the distally nested CRKL containing interval for major mental impairment, while the distally adjacent interval containing D22S308 and / or BCR is frequently associated with choanal atresia and usually atypical congenital heart defects such as arterial stenoses, as well as mild learning difficulties with the proximal part.

Although the incidence of atypical deletions was considered low and only probes covering the D22S75 or TUPLE1/HIRA loci are generally used for diagnostics, our results show, that by using the latter probes only, about 6 % of 22q11.2 deletions are missed. With respect to efficiency of labour, however, atypical deletions not detectable with the widely used commercial probes are rare in patients with conotruncal heart defects, but common in patients with atypical phenotypes only weakly overlapping with the DGS/VCFS spectrum.
Acknowledgements

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The study was approved by the University of Erlangen-Nuremberg ethical review board and appropriate informed consent was obtained from human subjects. In individuals shown by photographs written consent for publication of images were given by individuals or their legal guardians.

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References


Figure legends

Figure 1: Schematic overview over the DGS/VCFS 22q11.2 deletion region. Low copy repeats are depicted as shaded squares and labelled with LCR22-1 to 8, and ADU according to Edelmann et al.\textsuperscript{18} and Mc Dermid & Morrow\textsuperscript{38}, as well as A-D with reference to Shaikh et al.\textsuperscript{6}. FISH clones used for this study are depicted as black, grey and white boxes. Bars below the map depict deletion sizes found in this study and in reported cases (light grey)\textsuperscript{6-13, 15, 16, 39-41}. Note that exact deletion borders are sometimes only estimated in reported cases.

Figure 2: Facial appearance of patients with typical and atypical 22q11.2 deletions. In individuals shown by photographs written consent for publication of images were given by individuals or their legal guardians. A, B: Mild expression of the typical VCFS facial appearance in the sib pair with the common 3 Mb deletion (A) and the proximally nested 1.5 Mb deletion (B). C, D: Patient 77104 at age 6 years. Note small mouth, pointed nasal tip, mild ptosis and low set ears mildly suggestive of 22q11.2 deletion. E, F: Patient 07604 at age 7½ years showing narrow eyes, thin upper lip, mild hypertelorism and broad, high nasal bridge. G-I: Patient 31502 at age 8½ month showing slightly broad folding of helix, thin upper lip, and widely spaced nipples. J, K: Father of patient 31502 with the same deletion showing narrow eyes, somewhat flat nasal tip, thin upper lip, and low set ears.
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