

CLINICAL STUDY

The spectrum of parathyroid gland dysfunction associated with the microdeletion 22q11

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Abstract

Objective: Clinical features associated with microdeletion of chromosome 22q11 (del(22)(q11)) are highly variable. Increased awareness of this condition is needed among specialists such as endocrinologists to reduce diagnostic delay and improve clinical care. The purpose of this study was to describe the phenotype of patients with del(22)(q11), focusing on parathyroid gland dysfunction.

Design and methods: Charts of 19 patients, including one kindred of three, known to have del(22)(q11) diagnosed by fluorescence *in situ* hybridization (FISH) were reviewed from the register of the department of Medical Genetics. Major clinical features including hypoparathyroidism phenotype were collected.

Results: Parathyroid dysfunction was present in 8 out of 16 patients (50%). Six patients were diagnosed with overt hypoparathyroidism. Hypocalcemia manifested as laryngeal stridor within the first days of life ($n=3$), seizures in infancy ($n=1$) and adolescence ($n=2$). The connection between hypoparathyroidism and diagnosis of del(22)(q11) was belated at the median age of 18 years. One patient had presented with transient neonatal hypoparathyroidism, and one patient had latent hypoparathyroidism. Within the kindred family, the phenotype variability including that of parathyroid dysfunction was as marked as between unrelated individuals. Standard karyotype failed to detect the deletion in 15 out of 19 cases.

Conclusions: Abnormal parathyroid function in the del(22)(q11) ranges from severe neonatal hypocalcemia to latent hypoparathyroidism. Del(22)(q11) should be considered as a potential cause of hypocalcemia even in young adult. When suspected, the diagnosis requires investigation by FISH. Furthermore, long-term calcemia follow-up is needed in normocalcemic patients with del(22)(q11) because of the possible evolution to hypocalcemic hypoparathyroidism.

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Introduction

Microdeletion of chromosome 22q11 (del(22)(q11)) occurs with an incidence of 1 per 4000 live births (1), which places this disorder among the most common genetic syndromes. As a consequence of the microdeletion, there is a congenital failure in the development of the derivatives of various pharyngeal arches and pouches (2). Clinical features associated with del(22)(q11) are highly variable (3) and include one or more of the following main anomalies: congenital cardiac defects, hypocalcemia, immunodeficiency from thymic hypoplasia, palate anomalies and velopharyngeal dysfunction, cognitive impairment, and minor facial dysmorphism. Despite heterogeneous clinical presentations, the genotype is remarkably homogenous with deletions present in the 22q11 region. Approximately 90% of patients have a typical deleted region (TDR) of 3 megabases, which includes an estimated 30

genes, whereas 8% of patients have a smaller deletion of 1.5 megabases which contain 24 genes (2). Hypocalcemia is invariably due to hypoparathyroidism, as originally described by DiGeorge in 1965 (4) and documented by the aplasia or hypoplasia of the parathyroid glands at surgery or autopsy (5). In a very large European cohort of patients known to have del(22)(q11), hypocalcemia was noted in 60% of patients (6). Usually hypoparathyroidism manifests during the neonatal period (7). However, late-onset appearance of symptomatic hypocalcemia has been reported in adolescence and adulthood (8,9). Due to varying presentation and severity, the diagnosis of chromosome 22q11 deletion syndrome is still often delayed. Increased awareness and knowledge among the many specialists such as endocrinologists, who may encounter these patients, is needed to reduce the diagnostic delay and provide optimal clinical care. The purpose of this study was to describe the phenotype of a

series of patients with del(22)(q11), diagnosed at Nice University Hospital and especially to investigate the spectrum of parathyroid gland dysfunction.

Subjects and methods

We have retrospectively studied the charts of 19 patients, including one family of three individuals, known to have del(22)(q11) diagnosed by a fluorescence *in situ* hybridization (FISH) test at Nice University Hospital between 1994 and 2004. The cases were identified from a register held by the department of Medical Genetics and included patients followed in the departments of Pediatrics and Endocrinology of our hospital. The age at the diagnosis was set as the age when the FISH test was performed. Based on literature findings of the major clinical features of the 22q11 deletion, the following data were gathered from the patient's medical records: congenital cardiac defect, palate anomalies including cleft palate or velopharyngeal insufficiency, dysmorphic features, thymic hypoplasia noticed by the surgeon during the heart operation or history of recurrent infections, learning difficulties, behavioral abnormalities and hypoparathyroidism. Assessment of parathyroid dysfunction included detailed clinical history inquiring into symptoms of hypocalcemia and the following biological features: concentrations of total calcium (normal range 2.2–2.6 mmol/l), phosphate (normal range 0.8–1.45 mmol/l), ionized calcium (normal range 1.17–1.30 mmol/l), intact parathyroid hormone (PTH) assessed by IRMA (Nichols Institute, California) (normal range 10–65 ng/l). Data regarding parathyroid involvement were available for 16 patients. Biochemical evaluation had been performed in six patients because of clinical symptoms and in the ten others, merely because of the discovery of del(22)(q11). Metaphase cells from peripheral blood lymphocytes (PBL) were prepared for standard chromosome analysis according to conventional procedures. At least 16 RHG-banded metaphase cells were analyzed for each patient. FISH was performed on metaphase cells from PBL according to the manufacturer's recommendations using the DG/VCFS critical region probes D22S75 (Oncor, B-Biogene, Illkirch, France) or TUPLE 1 (Vysis, Abbott Molecular Diagnostics, Rungis, France) located at 22q11.2. Both probes were used together with the control probe, arylsulfatase-A (ARSA), at 22q13.3. For each patient, at least 20 metaphase cells were scored for both the D22S75 or TUPLE1 and ARSA signals. Deletions 22q11 were asserted for the D22S75 or TUPLE1 signals, but not the ARSA signals, which were consistently missing on one of the chromosome 22. Familial screening was done in 13 of 17 kindred: karyotype and FISH analysis were performed on PBL samples from the

parents and siblings of the index patient. Informed consent was obtained from parents or patients according to age and ability to assent.

Results

The mean age of patients (11 males, 8 females) was 17 ± 12 years when data were collected. Age at the diagnosis ranged from 7 days to 48 years (median 18 years). Nine patients were born before FISH became a routine diagnostic test. Eight patients (42%) had been diagnosed in adolescence or young adulthood. Familial occurrence of the deletion was confirmed in 2 out of 13 tested kindred. One case included three individuals in two generations; two brothers (20 and 18 years old) and their mother (48 years old). In the other case, the affected patient's mother had a 22q11 deletion detected by FISH but no clinical data were available for her; thus, she was not included in the study. In four cases, the del(22)(q11) was visible by using conventional R-banding karyotyping and was confirmed by FISH analysis. In 15 cases, the R-banded karyotypes were apparently normal (Fig 1A) whereas FISH studies demonstrated a deletion in the 'DiGeorge chromosomal region' (Fig 1B). Major clinical findings are reported in Table 1. Parathyroid dysfunction was present in 8 out of 16 patients (50%). Based on frank hypocalcemia with inappropriately low PTH concentration, six patients were diagnosed with overt hypoparathyroidism. Age at the diagnosis ranged from neonatal period to 14 years. Hypocalcemia manifested as laryngeal stridor within the first days of life ($n=3$), seizures in infancy ($n=1$) and adolescence ($n=2$). However, the connection between hypoparathyroidism and diagnosis of deletion 22q11 was belated at the median age of 18 years. These six patients were treated with 1,25-dihydroxyvitamin D and calcium supplementation. During adolescence, compliance to medication became poor in four patients due to behavioral disorders. They experienced recurrent general seizures and required intravenous treatment with calcium. One patient, the mother of two affected boys, was considered to have latent hypoparathyroidism. One patient, the elder son of the affected family, had presented with transient neonatal hypoparathyroidism. Symptoms of hypocalcemia and biochemical findings within the three affected kindred from this family are reported in Table 2, illustrating the variability of hypoparathyroidism phenotype. The mother had a normal total calcium (2.30 mmol/l) but a low PTH level (17 ng/l) compared to low ionized calcium concentration (1.10 mmol/l) and high phosphate concentration (1.78 mmol/l). She did not manifest any symptoms of hypocalcemia, but the brain CT scan showed multiple calcifications located in the basal ganglia and cerebellum. The elder son had presented with laryngospasm, associated with hypocalcemia in the neonatal period. Calcium supplementation was

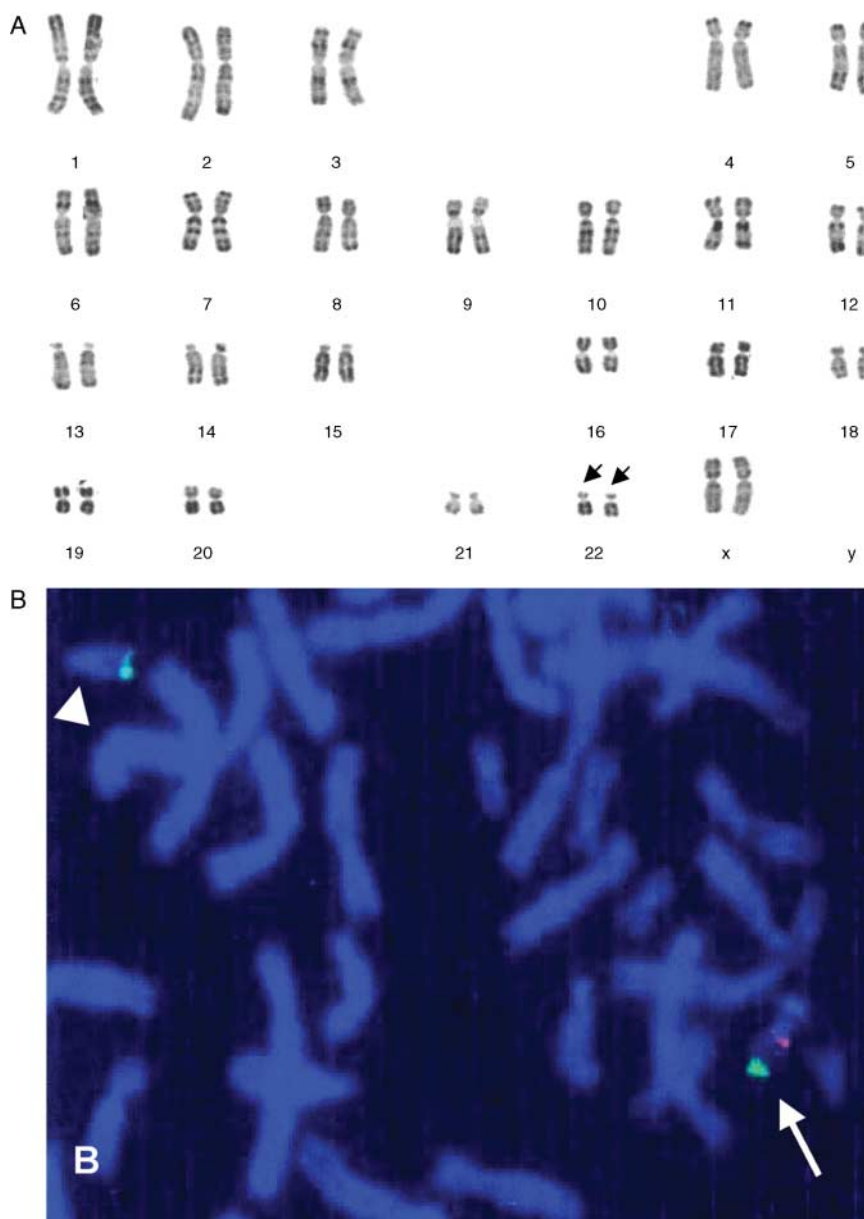


Figure 1 R-banded karyotype (A) and FISH analysis (B) on peripheral blood lymphocyte metaphase cell from patient. The microdeletion is detected by the FISH analysis only. (A) the karyotype is 46,XX. No anomaly is detected. The two chromosome 22s are apparently similar in size (arrows). (B) Both TUPLE1 (DiGeorge region in 22q11; red signal) and ARSA (control probe at 22q13.3; green signal) loci are present on the normal chromosome 22 (arrow) whereas only ARSA is present on the other chromosome 22 (arrowhead), indicating a 22q11 microdeletion.

discontinued at 3 months of age without recurrence of the symptom. Now 20 years old, he remains asymptomatic and has normal calcium, phosphate and PTH levels. The youngest son had generalized seizures at 14 years of age and was treated with an antiepileptic drug. The biochemical evaluation performed four years later revealed a severe hypocalcemia as demonstrated by total calcium (1.74 mmol/l) and ionized calcium (0.97 mmol/l) compared with undetectable serum concentration of intact PTH. Treatment of

hypoparathyroidism was initiated at that time. In addition, a congenital cardiovascular anomaly was confirmed in 11 patients. The most common defects were ventricular septal defect ($n=6$) and tetralogy of Fallot ($n=4$). Truncus arteriosus was observed in one case. All patients had dysmorphic features such as minor auricular anomalies (thick helices, small or round ears) and/or prominent tubular nose, small arch-shaped mouth, hooded eyelids and hypertelorism. Adolescent and adult patients showed more

Table 1 Parathyroid dysfunction and major clinical features of the 22q11deletion for all patients.

Sex	Age at diagnosis	Feature leading to diagnosis	Parathyroid dysfunction	Manifestations of hypocalcemia	Cardiac defect	Dysmorphic features	Palate anomalies	Learning difficulties	Behavior abnormalities
M	7 ^d	Hypoparathyroidism	+	Neonatal laryngeal stridor	-	+	-	+	-
M	8 ^m	Developmental delay	-	None	-	+	-	+	+
M	8 ^m	Cardiac defect	-	None	+	+	+	+	-
M	9^m	Cardiac defect	+	Neonatal laryngeal stridor	+	+	+	+	-
M	3 ^y	Developmental delay	-	None	+	+	+	+	-
M	3.5 ^y	Cardiac defect	NA	NA	+	+	-	+	-
M	6 ^y	Cardiac defect	-	None	+	+	-	+	+
M	18^y	Hypoparathyroidism	+	Seizures in adolescence	-	+	+	+	+
M	20^y	Cardiac defect	+*	Transient neonatal laryngo spasm	+	+	-	+	-
M	20^y	Hypoparathyroidism	+	Neonatal laryngeal stridor	-	+	-	+	+
M	20 ^y	Cardiac defect	NA	NA	+	+	+	+	+
F	7 ^d	Cardiac defect	NA	NA	+	+	-	+	-
F	7 ^d	Cardiac defect	-	None	+	+	-	-	-
F	7 ^d	Cardiac defect	-	None	+	+	-	-	-
F	2 ^y	Cardiac defect	-	None	+	+	-	+	+
F	15^y	Hypoparathyroidism	+	Seizures in adolescence	-	+	+	+	+
F	18^y	Hypoparathyroidism	+	Seizures in infancy	-	+	-	+	+
F	33 ^y	Abnormal behavior	-	None	-	+	+	+	+
F	48^y	Dysmorphic features	+ [§]	Intracranial calcifications	-	+	+	-	-

Patients with parathyroid dysfunction are in bold.

M, male; F, female; Age at diagnosis was set as the age when the FISH test was performed; m, months; y, years; d, day(s); NA, findings non available; +, clinical feature present; -, clinical feature absent; * transient hypoparathyroidism in neonatal period; [§]latent hypoparathyroidism.

pronounced facial anomalies than neonates and young children. Palate anomalies were reported in eight patients including submucous cleft palate ($n=3$), velopharyngeal insufficiency and hypernasal speech ($n=5$). Hypoplasia of the thymus was suspected in two cases because of the failure to detect the thymic tissue during surgical correction. Six patients had a history of recurrent pharyngo-tracheal infections. All but 3 of the 19 patients were considered to have developmental delay or learning difficulties. Behavioral abnormalities were reported in nine cases. Two patients had evidence of a psychiatric illness.

Discussion

Monoallelic microdeletion of chromosome 22q11 is considered as the most common human deletion syndrome. However, as shown in the population-based study in Western Sweden (1), the number of individuals diagnosed depends on the experience and awareness of the syndrome among specialists who encounter these patients, and also on the severity of the phenotype. Prior to the discovery of the deletion (10–12), many names

have been attributed to this disorder (DiGeorge syndrome, velo-cardiofacial syndrome, conotruncal anomaly face syndrome), illustrating the clinical variability of the phenotype associated with del(22)(q11).

In our series, parathyroid dysfunction was present in 50% of the patients. Accurate assessment of the prevalence of hypoparathyroidism in del(22)(q11) depends not only on the selection criteria used but also on recognition. Because mild or transient hypocalcemia may frequently be missed (13), a systematic screening is required for its detection. This was not done in our study. McDonald-McGinn *et al.* reported hypocalcemia in 77 of 158 (49%) patients with a confirmed del(22)(q11) (14). In a large European cohort, 60% of patients were hypocalcemic (6).

Hypoparathyroidism is most likely to present with symptoms of hypocalcemia, seizures, tremors or tetany in the neonatal period. Active transport of calcium from mother to fetus is abruptly interrupted with birth and calcium intake within the first few days of life is insufficient to maintain normal calcium levels in neonates with a reduced parathyroid reserve. In some children, most likely those with severe parathyroid

Table 2 Symptoms of hypocalcemia and biochemical findings within the three affected kindred from the same family.

Symptoms of hypocalcemia		Total calcium (mmol/l) (normal range 2.2–2.6)	Ionized calcium (mmol/l) (normal range 1.17–1.30)	Phosphate (mmol/l) (normal range 0.85–1.45)	Intact PTH (ng/l) (normal range 10–65)
Mother					
48 years	Intracranial calcifications	2.30	1.10	1.78	17
Elder son*					
20 years	Transient laryngospasm in neonatal period	2.30	1.17	1.30	31
Youngest son					
18 years	Seizures in adolescence	1.74	0.97	1.90	<8

*Index case.

defect, hypocalcemia is usually persistent, neonatal hypoparathyroidism being the primary manifestation of del(22)(q11). All infants with congenital hypoparathyroidism should be thoroughly evaluated for other physical features and screened for the del(22)(q11) by FISH. Symptomatic hypocalcemia may also manifest later in life (8–9,15). Two of our patients developed general seizures at 14 years of age and were treated with anticonvulsant therapy. The diagnosis of PTH-deficient hypocalcemia was initially missed and considered later on. Based on the history of learning disability and dysmorphic facial features, chromosome 22q11 deletion syndrome was suspected and confirmed by FISH. So, the diagnosis of del(22)(q11) should be entertained even in adolescents and adults with hypocalcemia (9). As observed in the literature (16, 17), we have also noted one case of transient severe neonatal hypocalcemia with spontaneous resolution during infancy. Recurrence of hypoparathyroidism may be precipitated during periods of increased metabolic demand such as cardiopulmonary bypass (18).

The del(22)(q11) typically occurs *de novo*, although it is inherited in about 10–20% of cases with a marked excess of maternally inherited deletions(14). Even within one kindred family, the phenotypic variability including that of parathyroid gland dysfunction, may be as marked as between unrelated individuals (8). As illustrated in this study by the three affected members from the same family, the spectrum of parathyroid gland dysfunction associated with del(22)(q11) ranges from symptomatic hypocalcemic hypoparathyroidism to normocalcemia with abnormally low basal PTH levels. This latent hypoparathyroidism may change over time and become apparent and symptomatic with increasing age or during the period of hypocalcemic stress such as infectious disease, pregnancy or surgery (8). The inability to increase PTH secretion appropriate for hypocalcemic stimulus can be demonstrated with a provocative test using disodium edetate infusion (8, 19). It is suggested that families with 22q11 syndrome should be informed of the symptoms that might occur with hypocalcemia (20). Moreover, screening of abnormal parathyroid function should be considered

in the regular follow-up of patients with del(22)(q11) (21).

Congenital cardiac defect is usually the first presenting symptom of del(22)(q11) in children, reported in about 60% of cases (22). This major clinical feature commonly leads to the diagnosis. In return, a high proportion of affected children have no cardiac defect and a risk of diagnostic delay (23) as illustrated by the late mean age at the diagnosis in our study (18 years). Cohen *et al.* (24) have reviewed the phenotype features of 126 adults with the del(22)(q11) (age at diagnosis > 18 years). They presented much lower rates of congenital heart defects, cleft palate and psychiatric disorders than those reported in children. On the other hand, cognitive impairment and facial anomalies may be the key findings that could help clinicians identify the syndrome in adults (24). The most recurrent symptoms shown by our patients were indeed typical facies and learning disabilities. Some facial anomalies, such as bulbous nasal tip, may become more apparent with age (25).

Finally, our study emphasizes that standard karyotype often fails to detect the deletion. When suspected, the diagnosis of del22q11 requires investigation by FISH analysis. It is therefore very important to make the clinical information concerning the patient available to the cytogenetics laboratory and to specifically request the 22q11 microdeletion to be searched by FISH. Moreover, it is worthy to ask for complementary FISH detection when patients had been tested by conventional karyotype only, particularly if this karyotype has been done before 1992 when FISH techniques were not routinely available.

Conclusions

The spectrum of parathyroid gland dysfunction associated with del(22)(q11) ranges from severe neonatal hypocalcemia to latent hypoparathyroidism. Del(22)(q11) should be considered as a potential cause of hypocalcemia even in young adult. Conversely, long-term follow-up should be given to patients with normocalcemia in the del(22)(q11) because of its

possible evolution to hypocalcemic hypoparathyroidism. Appropriate recognition and evaluation of individuals with del(22)(q11) are therefore essential to provide optimal clinical care. Endocrinologists should be aware of such common deletion and be involved in medical management and follow-up.

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