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A Study of the Clinical and Radiological Features in a Cohort of 93 Patients with a *COL2A1* Mutation Causing Spondyloepiphyseal Dysplasia Congenita or a Related Phenotype

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Type 2 collagen disorders encompass a diverse group of skeletal dysplasias that are commonly associated with orthopedic, ocular, and hearing problems. However, the frequency of many clinical features has never been determined. We retrospectively investigated the clinical, radiological, and genotypic data in a group of 93 patients with molecularly confirmed SEDC or a related disorder. The majority of the patients (80/93) had short stature, with radiological features of SEDC (n = 64), others having SEMD (n = 5), Kniest dysplasia (n = 7), spondyloperipheral dysplasia (n = 2), or Torrance-like dysplasia (n = 2). The remaining 13 patients had normal stature with mild SED, Stickler-like syndrome or multiple epiphyseal dysplasia. Over 50% of the patients had undergone orthopedic surgery, usually for scoliosis, femoral osteotomy or hip replacement. Odontoid hypoplasia was present in 56% (95% CI 38–74) and a correlation between odontoid hypoplasia and short stature was observed. Atlanto-axial instability, was observed in 5 of the 18 patients (28%, 95% CI 10–54) in whom flexion-extension films of the cervical spine were available; however, it was rarely accompanied by myelopathy. Myopia was found in 45% (95% CI 35–56), and retinal detachment had occurred in 12% (95% CI 6–21; median age 14 years; youngest age 3.5 years). Thirty-two patients complained of hearing loss (37%, 95% CI 27–48) of whom 17 required hearing aids. The ophthalmological features and possibly also hearing loss are often relatively frequent and severe in patients with splicing mutations. Based on clinical findings, age at onset and genotype–phenotype correlations in this cohort, we propose guidelines for the management and follow-up in this group of disorders.

Key words: spondyloepiphyseal dysplasia; SEDC; genotype–phenotype; review; *COL2A1*

INTRODUCTION

Type 2 collagenopathies result in a spectrum of lethal and non-lethal skeletal dysplasias. The former includes achondrogenesis type 2, hypochondrogenesis, and platyspondylic dysplasia Torrance type, while the latter encompasses Stickler syndrome, spondyloepiphyseal dysplasia congenita (SEDC), spondyloperipheral dysplasia, Kniest dysplasia, SED with metatarsal shortening (formerly

Czech dysplasia), and SED with pronounced metaphyseal changes (including SEMD Strudwick type).

Although Type 2 collagen disorders are relatively common in a clinical genetics setting, there have been very few publications based on large cohorts delineating the phenotype and clinical course [Nishimura et al., 2005]. However, it is well established that palatal abnormalities can be associated and patients with the non-lethal disorders can develop age-related orthopedic, spinal, ocular, and hearing problems.

Atlanto-axial instability due to odontoid hypoplasia and/or the presence of lax ligaments, has been reported in many patients with SEDC. Odontoid hypoplasia has been estimated to occur in up to 80% of patients although in older studies the diagnosis of SEDC was not molecularly confirmed [Svensson and Aaro, 1988; Skeletal Dysplasia Group, 1989; Takeda et al., 1991; Nakamura et al., 1998; Miyoshi et al., 2004; Nishimura et al., 2005; Ain et al., 2006; Veeravagu et al., 2012]. The percentage of patients suffering from myelopathy has not been well defined. Marked short stature with a height below −7 SD and severe coxa vara have both been reported as high-risk factors for cord compression [Skeletal Dysplasia Group, 1989; Nakamura et al., 1998; Miyoshi et al., 2004].

Ophthalmological complications such as severe myopia, vitreous anomalies and perivascular lattice degeneration of the retina are well recognized to occur in a significant subset of patients with SEDC and Kniest dysplasia [Hamidi-Toosi and Maumenee, 1982; Ikegawa et al., 1993; Spranger and Wiedemann, 1966]. Hearing loss has also frequently been reported, often without any details about the type of hearing deficit (conductive, perceptive or mixed) [Bogaert et al., 1994; Gilbert-Barnes et al., 1996; Nishimura et al., 2005; Zhang et al., 2011].

We have previously reviewed the clinical features of patients with Stickler syndrome due to truncating *COL2A1* mutations [Hoornaert et al., 2010]. In the current study, we attempt to gain insight into the clinical features and complications in non-lethal type 2 collagen disorders other than Stickler syndrome, based on 93 molecularly confirmed patients with SEDC or a related phenotype. The growth data obtained in this cohort have been reported separately [Terhal et al., 2012]. We searched for genotype/phenotype correlations and compared our results with previous observations described in the literature. The ultimate goal was to provide evidence-based guidelines for the management and counseling of this group of patients.

MATERIALS AND METHODS

Study Design, Inclusion, and Exclusion Criteria

The study was approved by the Institutional Review Board of the University Medical Centre Utrecht. The patients were recruited through three laboratories that are performing DNA analysis of *COL2A1*. The study population included patients with a heterozygous *COL2A1* mutation. Patients with loss-of-function mutations (resulting in Stickler syndrome) were excluded as well as patients with a perinatally lethal phenotype (achondrogenesis type 2/hypochondrogenesis and platyspondylic dysplasia Torrance type). After written informed consent was obtained from the patient or his/her responsible relatives, the referring physician was asked to complete a checklist (Supplementary File V) and to send radiographs (radiograph of the hand, feet, arm, spine, pelvis, and a flexion/extension X-ray of the cervical spine), preferably taken at different ages.

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Osteoarticular Data

The available radiographs were evaluated by a radiologist (R.N.) and clinical geneticists (G.M., P.T.) with experience in skeletal dysplasias. The radiological criteria for the diagnosis were based on several standard textbooks [Lachman and Taybi, 2007; Spranger et al., 2012]. For example, coronal clefts, metaphyseal widening, large epiphyses, prominent joints, or dumbbell-shaped femora in infancy were considered as indicative features for Kniest dysplasia. As suggested before by Wynne-Davies et al. and Nishimura et al., we used the severity of the coxa vara as a parameter to assess the severity of the SEDC (SEDC-M, mild or SEDC-S, severe) [Wynne-Davies and Hall, 1982; Nishimura et al., 2005]. We also took into account the presence or absence and severity of the scoliosis. Patients were classified as having a "severe" scoliosis if they had a Cobb angle of $\geq 30^{\circ}$ or if surgery was performed to correct the spinal deformity. If there was no radiograph of the spine available, the clinical assessment

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of the referring physician alone was used to classify the scoliosis ("severe" or "mild").

Dens Hypoplasia and Atlanto-Axial Instability

Plain radiographs and flexion-extension films of the lateral cervical spine were evaluated for the presence of odontoid hypoplasia and atlanto-axial instability. Odontoid hypoplasia was said to be present when the upper tip of the dens did not reach the upper border of the vertebral body of the atlas. If the child was very young, the diagnosis of odontoid hypoplasia was made in comparison with age-matched controls illustrated in the standard textbooks [Kahn et al., 2007; Swischuk, 2002]. Atlanto-axial instability was diagnosed if the atlas-dens interval (distance between the odontoid process and the posterior border of the anterior arch of the atlas) was greater than 3 mm in adults or greater than 5 mm in children [Nakamura et al., 1998; Swischuk, 2002]. Where radiographs of the cervical spine were not available, we used the written information provided by the referring physician (in Supplementary Table SIII marked by a "Q," questionnaire). The difference in mean height between patients with dens hypoplasia and patients with a normal dens was tested with a Student's *t*-test.

Ophthalmological Data

We asked the referring physicians the results of the most recent eye examination (with an indication of age where known). Myopia was subdivided into mild myopia (between -1 and ≤ -3 Dpt), moderate myopia (> -3 and ≤ -6 Dpt) and severe myopia (> -6 Dpt). If no quantitative data were given about the degree of myopia, the description of the physician in the checklist was used (for example, "mild" or "high"). We combined the clinical and ophthalmological data of the patients in Supplementary Table SIII.

Hearing Data

We asked the referring physicians whether patients had complaints of hearing loss, and to specify the type and severity of the hearing loss (Supplementary File V). All retrieved audiograms were evaluated by an otolaryngologist. If audiometric thresholds were incomplete or patients were too young to perform audiometry, other investigations were evaluated (free field examination, auditory brainstem response) to assess the levels of hearing loss. In patients for whom no audiogram was available, we did not specify the type and severity of the hearing loss since the information provided was not always reliable. Hearing loss was defined as the average loss at 0.5, 1.0, and 2.0 kHz of 20 dB or more in at least one ear. The severity of the hearing loss was defined as the average threshold level at 0.5, 1, and 2 kHz according to the definition of Clark [1981]. We combined the clinical and audiometric data of patients with hearing loss in Supplementary Tables SIII and SIV.

RESULTS

Of 151 checklists, 93 (62%) were completed and returned by the referring physician. Most patients were living in the Netherlands (29 patients), the United Kingdom (22 patients), and France (11 patients),

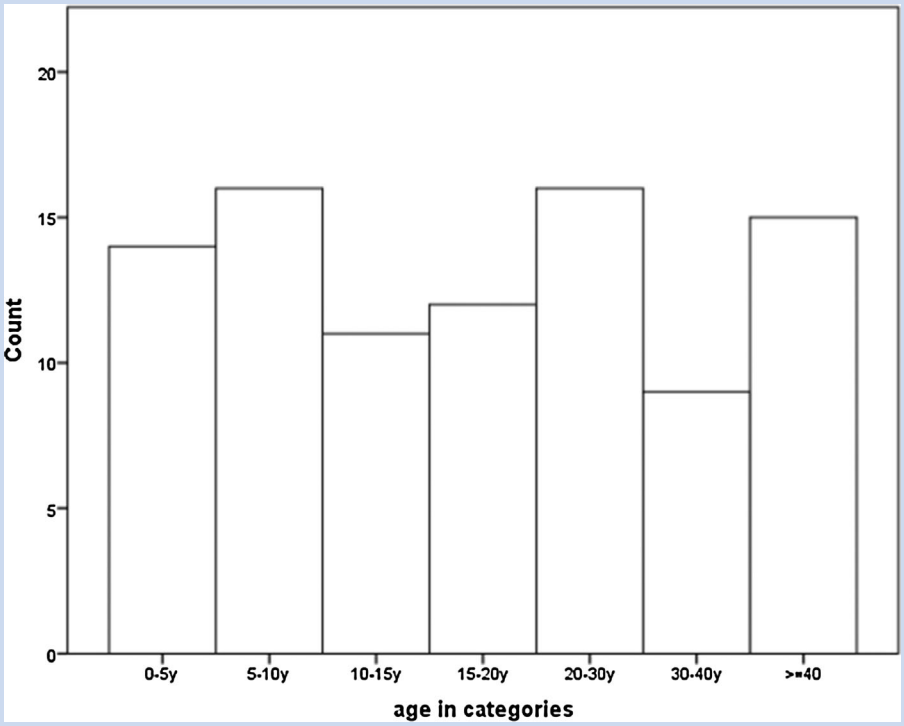

FIG. 1. Number of patients according to age category (in years).

but other countries were also represented (Supplementary Table SI). The study group included 51 (55%) females and 42 (45%) males. The median age of the patients was 17 years (range 4 months to 70 years; interquartile range, IQR 7.2–31.6 years, Fig. 1). An overview of the clinical features and complications in our study group is given in Supplementary Table SII. The clinical and molecular data of individual patients are presented in Supplementary Table III.

Mutations

Of the 93 patients, 68 (73%) were heterozygous for missense mutations resulting in a glycine substitution in the triple helical domain of the type 2 procollagen chain. Glycine to serine substitutions were the most common mutations, followed by glycine to aspartic acid and glycine to arginine mutations. (Table I). In 47 of the 93 patients (51%) the mutation was de novo. In 31 patients (33%) the mutation was inherited from one of the parents, one mother being a gonadal mosaic for the mutation. In the remaining patients this information was not available.

Neonatal Manifestation and Development

Nine percent of the patients were born before 36 weeks gestation, 72% at or after 36 weeks, and in 19% this information was not available. The mean birth length of the neonates born at or after 36 weeks gestation was 44.5 cm (standard deviation, SD = 3.0 cm). Cleft palate was seen in 20 patients (22%, 95% CI 14–32), which was a submucous cleft in one. In two patients, a bifid uvula was present. Pierre Robin sequence was seen in 13 of 20 neonates with cleft palate. Twenty-two neonates had respiratory problems (26%, 95% CI 17–37). In eight patients this was related to Pierre Robin sequence, requiring tracheostomy in two of them. Other reasons for respiratory distress were tracheomalacia, bronchomalacia, a small chest or pneumonia. Two patients were prematurely born and developed respiratory distress syndrome.

Almost all patients had normal cognitive development. There were only two exceptions, one patient who had a respiratory arrest at age two years due to respiratory syncytial virus infection, and another patient who had a speech delay that was probably related to hearing loss. Motor development was delayed in 34% (95% CI 24–46) of the

TABLE I. Number of Patients According to Mutation Group

Mutation type	Frequency
splice	9
glycine to serine	28
glycine to aspartic acid	12
glycine to valine	9
glycine to arginine	12
glycine to cysteine	4
glycine to glutamine	3
C-terminal propeptide	6
arginine to cysteine	5
deletion or duplication in triple helix	5
Total	93

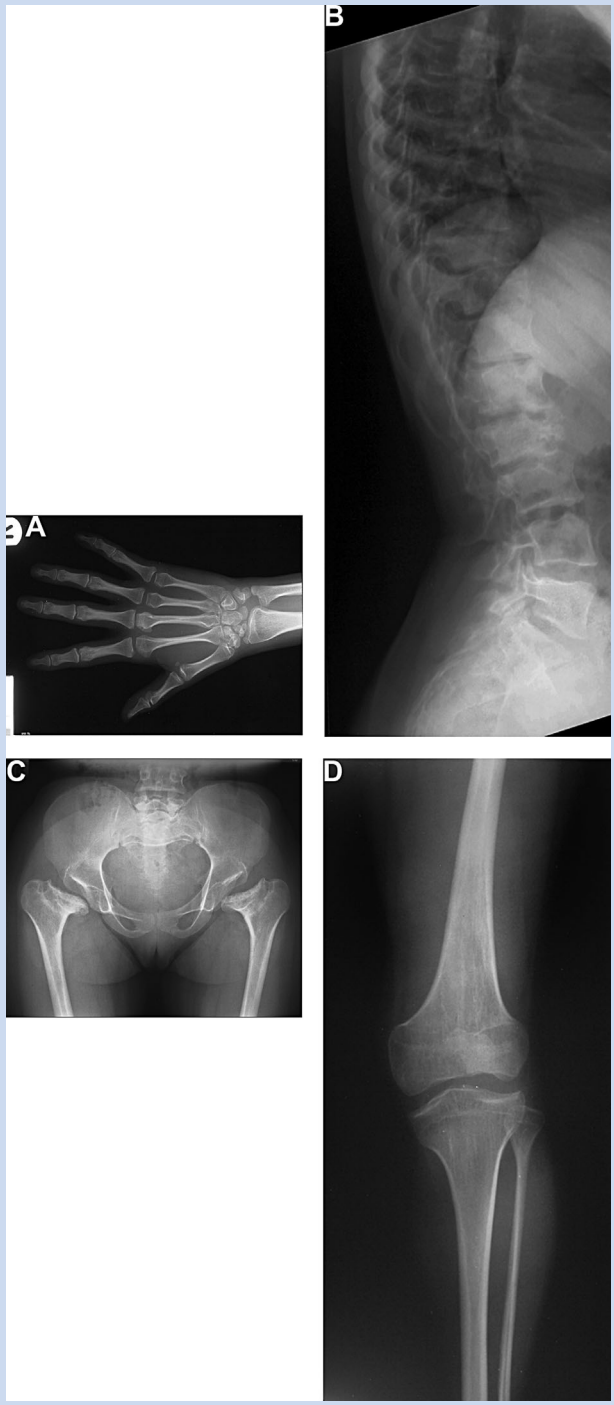

FIG. 2. Typical radiological picture of patient 34 with SEDC due to the p.Gly375Asp mutation at adult age. A: Marked flattening of the caput of metacarpal bones 2–5, fragmentation of the os scaphoideum and os trapezium, shortening of the carpus and ulna minus. B: Flattening of the vertebral bodies with irregular endplates, most pronounced at the anterior part of the vertebral body L2-L3. C: Dysplastic acetabulae, absent ossification of the proximal femoral epiphyses, broadening and irregularity of the femoral metaphyses with shortening of the collum and coxa vara. D: Mild flattening of the femoral condyles and sloping of the medial tibial condyle.

patients. Median age at walking was 18 months (IQR 15–23 months) (based on data from 56 patients).

Skeletal Phenotypes

Sixty-four patients were diagnosed as having SEDC. Figures 2 and 3 show radiographs of an adult patient with SEDC. Fifty-three of the SEDC patients had glycine substitutions in the triple helical domain of the type 2 collagen chain. Three patients in the SEDC group possessed p.Arg989Cys mutations, two had splicing mutations, four had duplications in the triple helical region and two had C-terminal propeptide mutations.

Five patients had SED with marked metaphyseal changes diagnosed as SEMD Strudwick type (Fig. 4). Four of them had a glycine substitution (p.Gly519Ser; p.Gly1005Ser; p.Gly1053Val; p.Gly1122Arg) and the remaining patient had a splicing mutation.

Seven patients were diagnosed as having Kniest or Kniest-like dysplasia (early arthropathy with progressive stiffness resembling pseudorheumatoid dysplasia). Six of these patients had a splicing mutation, and one an in-frame deletion in the triple helical region.

PLSD Torrance-like phenotypes with spikes at the ends of long bones were seen in two patients with a C-terminal threonine substitution (Fig. 5). In two other patients, carrying a C-terminal frameshift mutation, clinical findings were compatible with spondyloperipheral dysplasia (SEDC with mild brachydactyly, Fig. 6).

Thirteen patients had milder skeletal phenotypes. Four of them, the patients with the most amino-terminal located glycine substitutions, had normal stature and severe arthropathy with ocular involvement (patients with the p.Gly210Glu and p.Gly240Cys mutation) or normal radiographs with a diagnosis of Stickler syndrome (Family 8 with the p.Gly315Val mutation). Nine of them had a skeletal phenotype consistent with premature osteoarthritis (Family 110 with the p.Arg719Cys mutation) or mild SED resembling MED/Perthes at a younger age (Families 141 and 128 with the p.Gly744Ser and the p.Gly945Ser mutation respectively, Fig. 7).

Orthopedic Data

Clubfoot was found in 9% (95% CI 4–18) of the patients at birth. Interestingly, two unrelated patients with the same mutation (c.905C>T, p.Ala302Val) both presented with clubfoot deformity. Seventy-six percent (95% CI 65–84) experienced joint pain with a median age of onset at 8.8 years (IQR 4.1–12.0 years). Hips and knees were most frequently affected, but patients also suffered from pain in other joints, including shoulders, elbows, hands, feet, and ankles. Twenty-eight percent (95% CI 19–39) had pectus carinatum, and 7% (95% CI 3–15) a pectus excavatum. A scoliosis was present in 48% (95% CI 37–59) and was severe in just over one-third. In four patients with severe scoliosis, the ages at onset were

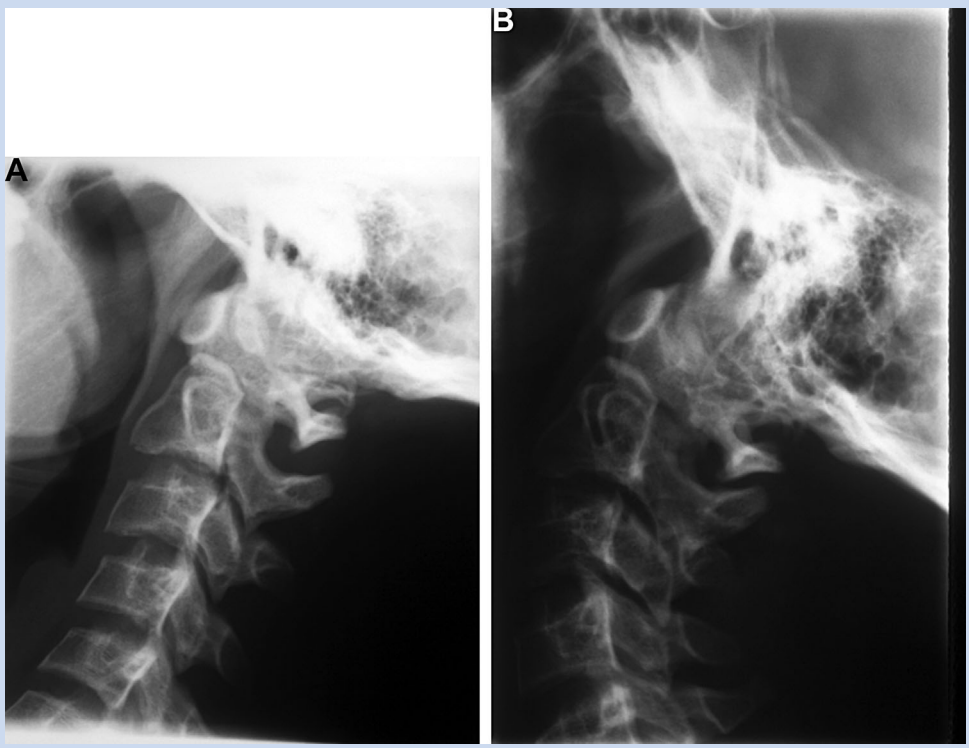

FIG. 3. A: Lateral radiographs of the cervical spine in flexion of patient 98 with SEDC due to the p.Gly351Val mutation at adult age. Note the severe dens hypoplasia. B: Lateral radiographs of the cervical spine in extension of patient 98. Note minimal posterior movement of C2 in relation to C1 (less than 5 mm, no atlanto-axial instability).

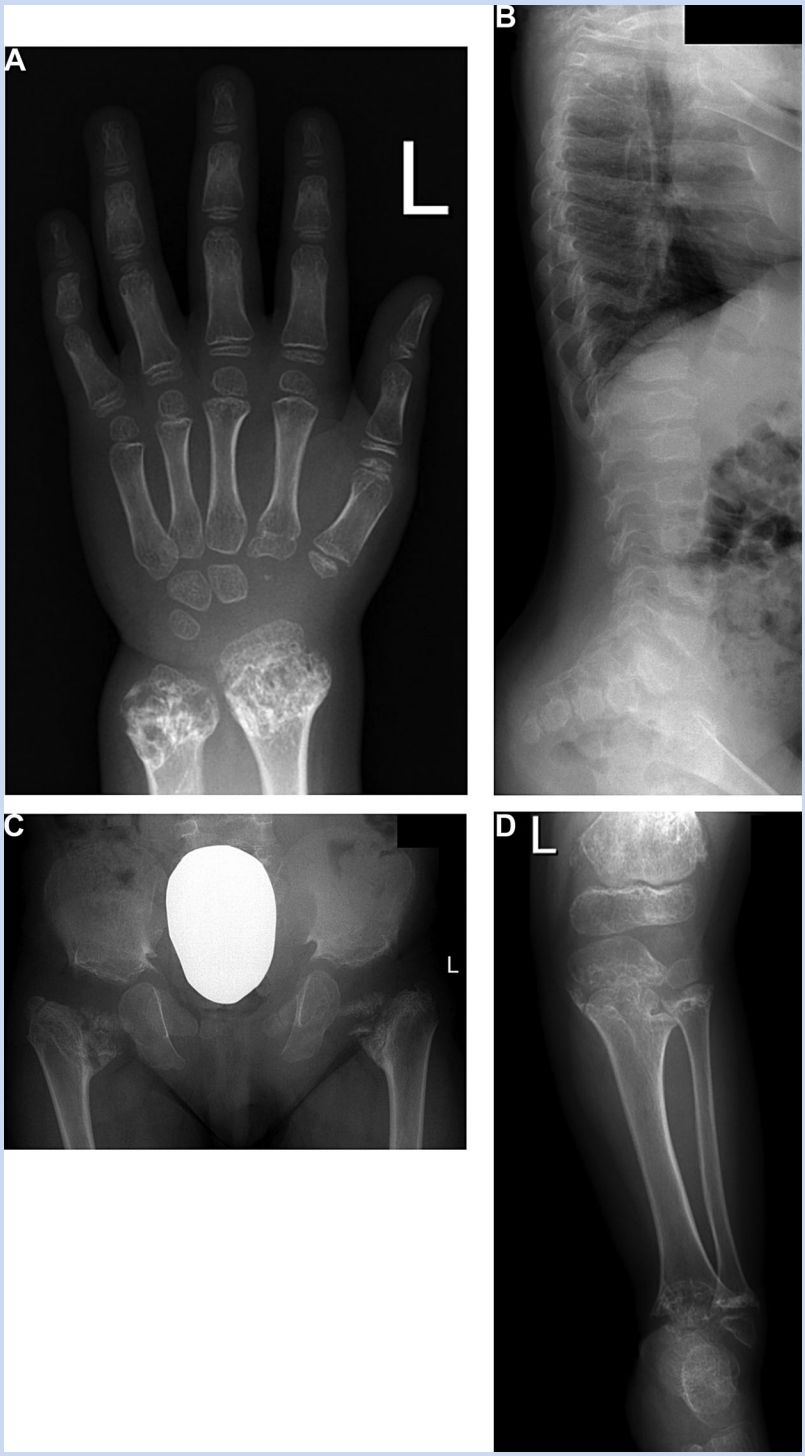

FIG. 4. SEMD Strudwick type in patient 10 with the p.Gly1122Arg mutation at the age of 6.8 years. A: Marked irregular ossification and broadening with radiolucencies of the distal metaphysis of the radius and ulna. Pseudoepiphysis of the distal first metacarpal bone and proximal second metacarpal bone. Generalized broadening and shortening of the phalanges. B: Flattening of the vertebral bodies with irregular endplates. C: Short, broad pelvic wings with horizontal acetabular roofs and narrow sciatic notches, severe coxa vara, severe broadening and irregularity of the fermoral metaphyses with corner fragments and almost completely absent collum, severe irregular ossification of the proximal femoral epiphyses. D: Irregular ossification of the distal femoral metaphysis, especially at its outer surface. Marked irregularity of the metaphyses of the proximal and distal tibia and fibula with deformed epiphyses.

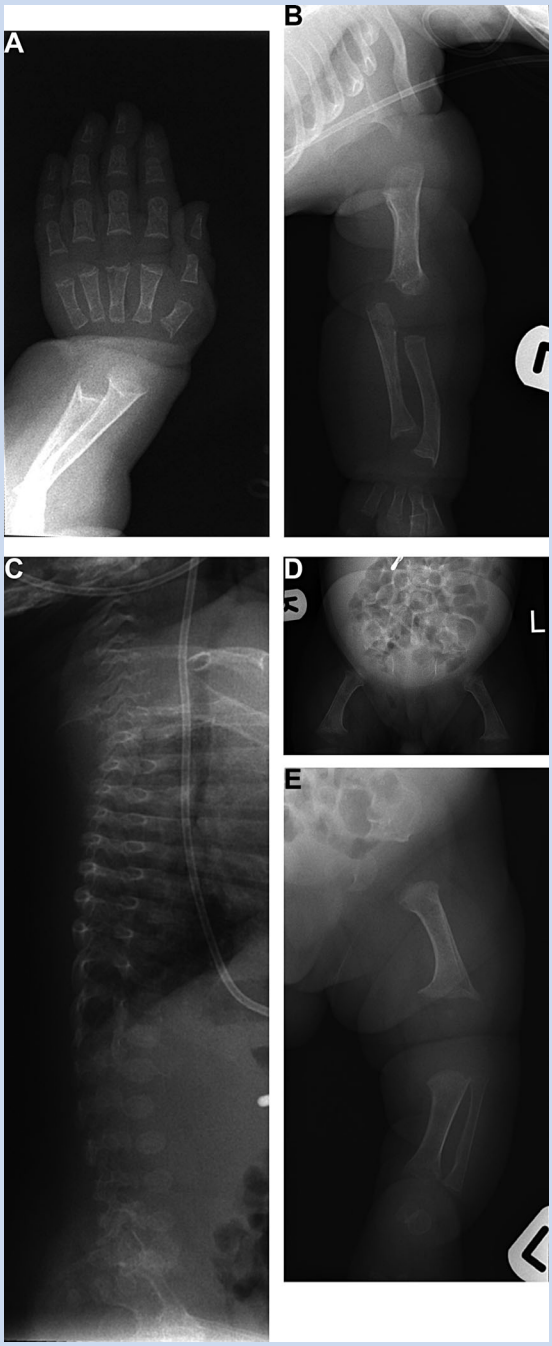

FIG. 5. Torrance-like radiological picture in patient 3 with the C-terminal p.Thr1322Ile mutation at the age of several months. A: Short and broad metacarpal and phalangeal bones, with broadening and cupping of the metaphyses. Spiking of the proximal metaphysis of the first metacarpal bone and of the distal metaphyses of the metacarpal bones 2–5. B: Short, squat bones of the left arm with broadening of the metaphyses and spiking of the distal radius and ulna. C: Ovoid vertebral bodies with mild beaking of L1. D: Short and broad pelvic bones with horizontal acetabular roofs and broad metaphyses of the proximal femora. E: Short, squat bones of the left leg with broadening of the metaphyses.

being recorded, as 3, 7, 8, and 13 years, respectively. The relation between genotype and the severity of coxa vara (which could be assessed in 46 patients) and scoliosis is shown in Figure 8A,B, respectively. Although it was sometimes difficult to compare X-rays at different ages, the severity of the coxa vara tended to be consistent among affected relatives in familial cases.

In this series 54% (95% CI 43–64) underwent orthopedic surgery. Eight patients had correction of clubfoot, eight patients underwent surgery to correct scoliosis and 30 patients had surgery on the lower limbs (osteotomies, epiphysiodesis), femoral osteotomy being the most common type of surgery in childhood. In adulthood (after the age of 20 years) hip replacement is the most common type of surgery. Median age at first surgery (omitting clubfoot surgery) was 10.5 years (range 20 months to 46 years; IQR 6–17 years). Ten patients needed to use a wheelchair permanently or daily.

Dens Hypoplasia and Atlanto-Axial Instability

Odontoid hypoplasia could be assessed in 32 patients and was present in 56% (95%CI 38–74). In 18 patients flexion-extension films of the cervical spine were available. Five of these patients (28%, 95 % CI 10–54) had atlanto-axial instability. Each of them also had dens hypoplasia. Most patients with atlanto-axial instability didn't have clinical symptoms. However, two patients had pain during neck flexion or extension, one patient had a torticollis and one patient had symptoms of "cervical myelopathy" in adulthood. Other cervical spine problems in our cohort included spondylolisthesis of C4 on neck flexion (one patient), anterior slip of C6/C7 and C3/C4 on flexion (one patient) and disc herniation at C5–C6 (one patient). Two patients underwent surgery, one because of atlanto-axial instability and the other because of spinal stenosis. In two other patients' surgery for stabilization of the cervical region is being considered.

In our series, dens hypoplasia was seen in all patients whose height was below the 0 SD on the SEDC growth chart (Fig. 9) but it was also found in some patients with heights above the mean or even at the upper limit of the SEDC growth chart. The mean height of 14 patients with radiologically confirmed dens hypoplasia was significantly lower than the mean height of nine patients with a normal dens (-0.22 SD versus $+1.12$ SD on the SEDC growth curve, $t = 3.04$, $df = 21$, $P = 0.006$).

Ophthalmological Data

Forty-five percent (95% CI 35–56) had myopia, which was moderate or severe in over half. In eight patients, the referring physician reported that the myopia was progressive. We were able to document the progression of the myopia in more detail in Patient 75 and Patient 92, with the Gly396Val and the Ala302Val mutation, respectively. Eye examination shortly after birth (at 4 months in patient 75 and at 6 weeks in patient 92) was normal while both patients had a myopia exceeding 10 Dpt at 6 years of age. Retinal detachment occurred in 12% (95% CI 6–21). In seven patients, retinal detachment was unilateral but in many of them preventive laser treatment was performed in the other eye. The median age at which the retinal detachment was detected was

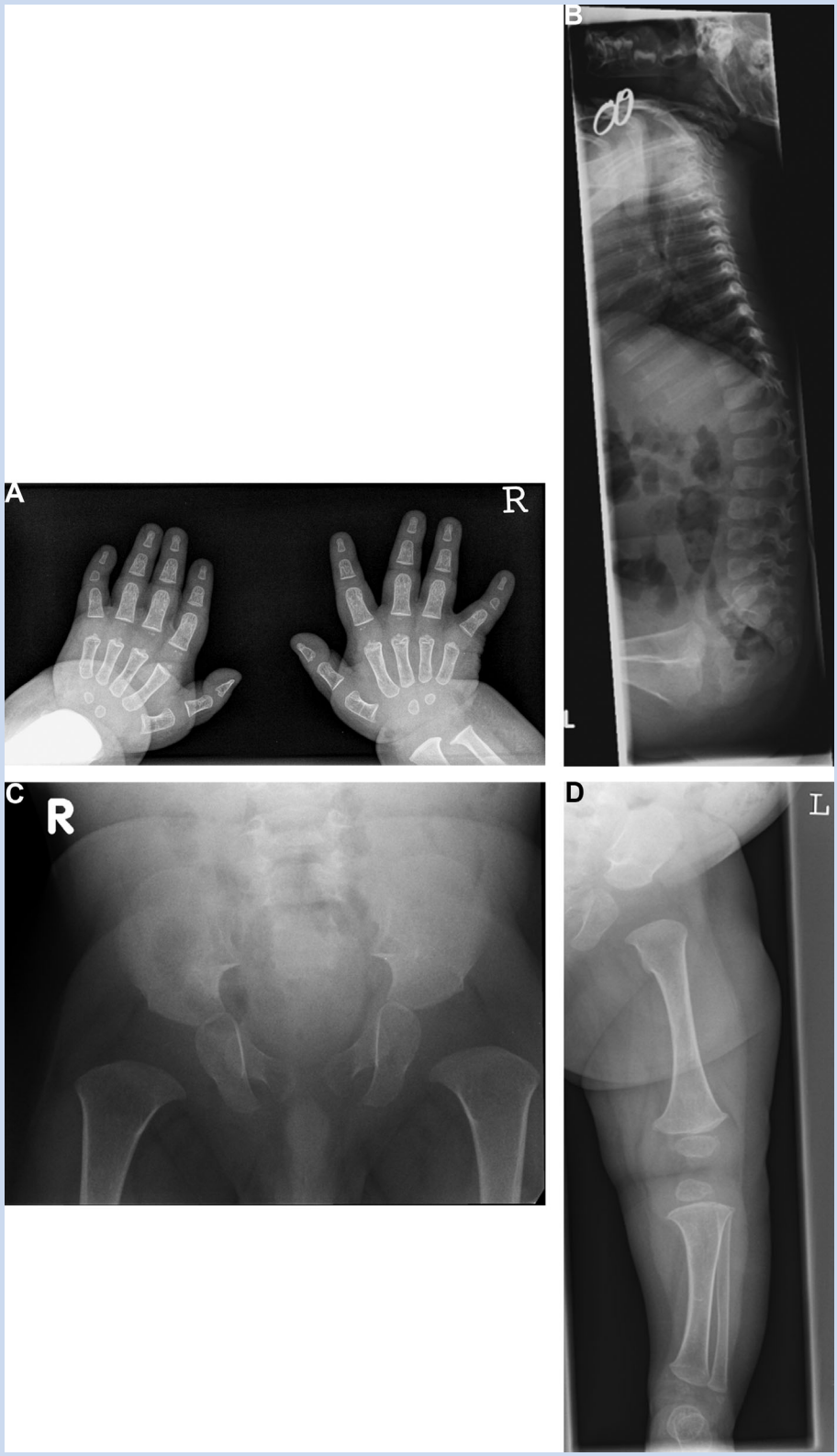

FIG. 6. SEDC radiological pattern with brachydactyly in patient 127 with the C-terminal c.4311delC mutation. A: Short and broad metacarpal and phalangeal bones at age 11 months. B: Normal appearance of the vertebral bodies at age 1.2 years. C: Mild shortening and broadening of the pelvic bones. Delayed ossification of the femoral epiphyses and broadening of the proximal femoral metaphyses at age 1.2 years. D: Mild shortening and broadening of the bones of the left leg, mild broadening of the metaphyses at age 11 months.

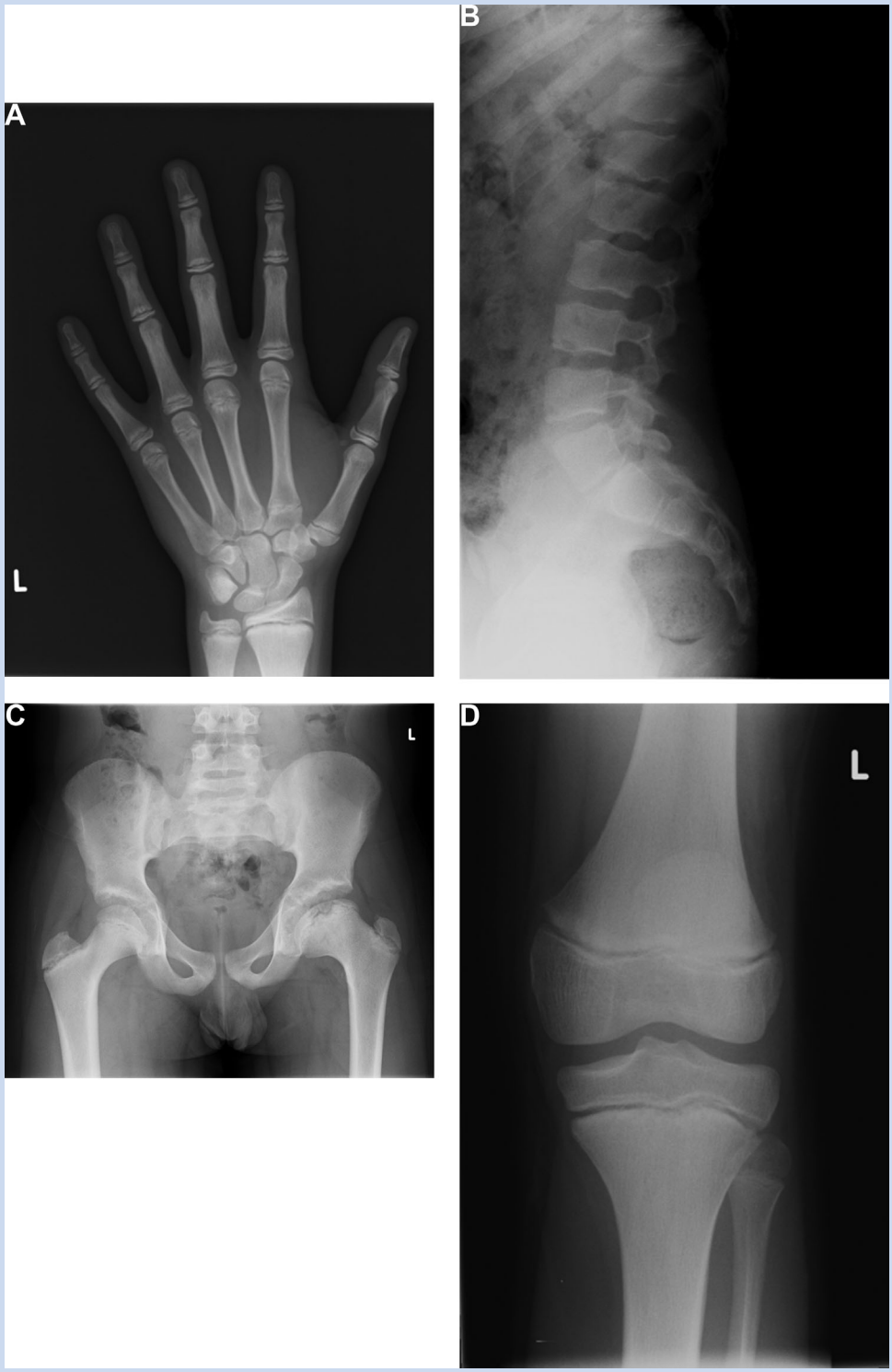

FIG. 7. MED/Perthes-like disease in patient 141b with the p.Gly744Ser mutation. A: Normal radiological appearance of the hand at age 14.8 years. B: Mild flattening and irregularities of the endplates of the thoracolumbar vertebral bodies at age 14.8 years. C: Radiograph of the pelvis at age 12.5 years. Small and somewhat flattened epiphysis of the right femoral head, severe broadening and flattening of the epiphysis and broadening of the metaphysis of the left femoral head after avascular necrosis. D: Normal appearance of the knee at age 12.5 years.

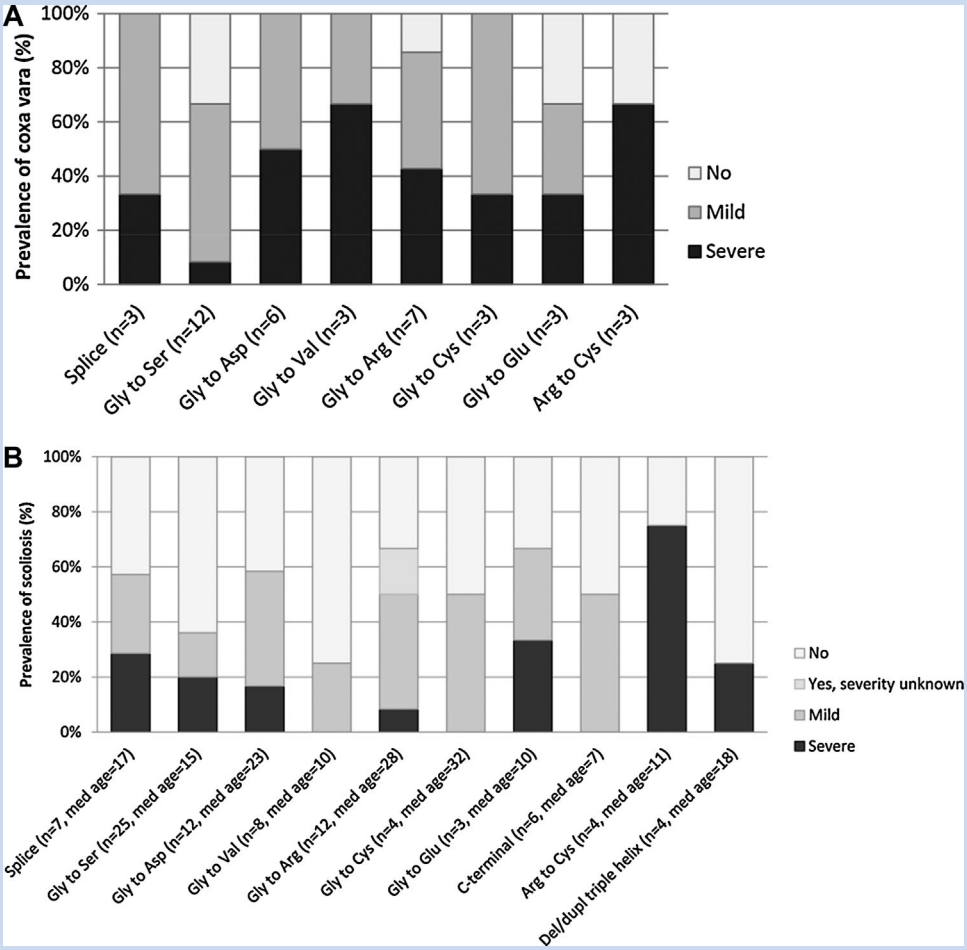

FIG. 8. A: Presence and severity of coxa vara, according to mutation group, n = number of patients from which information is available. B: Presence and severity of scoliosis according to mutation group. Med. age is median age of the patients from which information was available.

14.0 years (range 3.5–45 years; IQR 9.5–19.5 years). In the patient diagnosed at 3.5 years the retinal detachment was believed to have occurred earlier. The severity of myopia and presence or absence of retinal detachment in the different mutation groups is shown in Figure 10A,B. Patients with splicing mutations appear to have the most severe ophthalmological features. Eight of nine patients with a splicing mutation had myopia, and it was severe in six. Three of the nine patients with splicing mutations experienced a retinal detachment, including the child diagnosed at 3.5 years. Four patients with the most amino-terminal located glycine substitutions (p.Gly210Glu, p.Gly240Cys, p.Gly315Val), had ocular involvement.

Vitreous anomalies and cataract were reported in 16% (95% CI 9–27). However, the proportion of patients with vitreous anomalies may have been underestimated because of difficulties assessing the vitreous in children. Vitreous anomalies were observed without myopia and even with hypermetropia. Descriptions of the abnormal vitreous included "collapsed vitreous" and "aspecific fibrous degeneration." One patient had "posterior synechiae with vitreous opacities" (Supplementary Table SIII). Cataract was reported in 19% (95% CI 11–29), the youngest patient being 6 years old. One patient in our study was diagnosed as having congenital buphthalmos.

Hearing Data

Information about complaints of hearing loss was available in 87 patients. Thirty-two patients were recorded as complaining of hearing loss (37%, 95% CI 27 –48). Seventeen patients used hearing aids (of whom six had a splicing mutation). In 22 patients, we received the results of quantitative or semi-quantitative auditory examination comprising audiograms in 17 patients, free field testing in three patients and auditory brainstem responses (ABR) in two patients, although we did not get the raw ABR data. Of these 22 patients, 16 had complained of hearing impairment, but three patients were asymptomatic despite having mild or slight hearing loss during audiometry or free field testing. The type and severity of hearing loss in relation to the different mutations is presented in Supplementary Tables SIII and SIV.

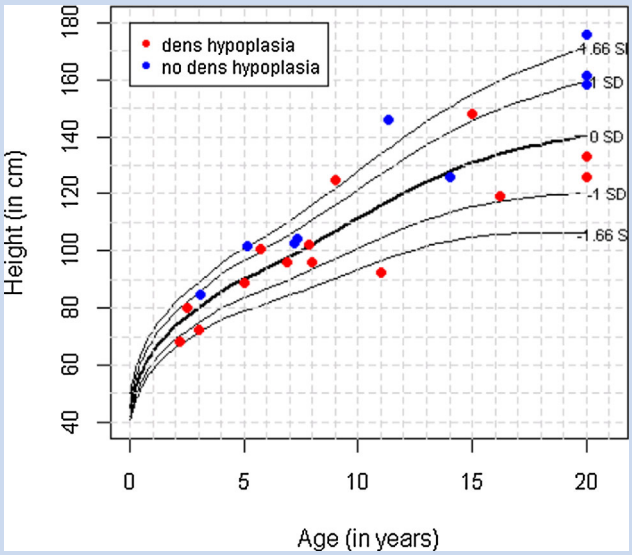

FIG. 9. The last measurement of height displayed on the SEDC growth chart in patients with X-ray confirmed dens hypoplasia (in red) or a normal dens (in blue).

DISCUSSION

In this retrospective study, we provide clinical data on a large group of 93 patients with SEDC or a related phenotype with confirmed mutations in the *COL2A1* gene. Due to allelic heterogeneity the number of patients with each mutation type was relatively small. In addition, the manifestation of many complications was age-dependent. Therefore, statistically significant genotype–phenotype correlations were not possible. Nevertheless, because this cohort included a number of patients with recurrent mutations and many families with affected siblings at different ages, we were able to gain insight into the spectrum and variability of the clinical features. We provide information regarding the frequency, severity, and age at onset of the major clinical problems, including skeletal phenotypes, orthopedic problems (scoliosis, club foot), ophthalmological abnormalities (myopia, retinal detachment), and hearing impairment.

Genotype-Skeletal Phenotype

For patients with a glycine substitution in the triple helical domain, there was no clear linear amino-to-carboxyterminal gradient in the severity of the radiological phenotype, contradicting the "gradient model" of disease severity previously postulated in collagen-related disorders such as osteogenesis imperfecta [Byers, 1990; Rauch et al., 2010]. For example, we identified several families, in whom a glycine to serine substitution at amino acid positions 744 and 945 (p.Gly744Ser and p.Gly945Ser, respectively) caused a relatively mild form of SED, even resembling MED, whereas a similar mutation nearby, at amino acid position 702 (p.Gly702Ser), resulted in severe SEDC. Our observation recapitulated the findings of Nishimura et al., who concluded that patients with glycine to serine substitutions can have either SEDC with mild or severe coxa vara, depending on the exact localization of the mutation. Furthermore, the nature of the substituting amino acid was important for the skeletal phenotype. Consistent with the results by Nishimura et al., we found that coxa vara tended to be more severe in patients with glycine to non-serine substitutions than patients with glycine to serine mutations. However, in our study, patients with the p.Gly504Ser or p.Gly1197Ser mutation were not as mildly affected as those reported by Nishimura et al., since some of them also showed severe coxa vara and scoliosis [Nishimura et al., 2005]. Our observation that the three most N-terminally located glycine substitutions caused SED phenotypes with (low) normal height, or Stickler-like syndrome, supported the observation by Hoornaert et al. that short stature phenotypes do not occur N-terminal to the glycine at position 303 and that specific missense mutations can cause Stickler syndrome instead of SEDC [Hoornaert et al., 2010].

Dens Hypoplasia and Atlanto-Axial Instability

We observed a statistically significant correlation between height and dense hypoplasia. All patients with a height below 0 SD on the SEDC growth chart had odontoid hypoplasia. A relationship between the presence of myelopathy due to atlanto-axial instability, severe short stature and severe coxa vara was previously reported by Nakamura et al. [1998]. However, the number of patients with myelopathy in our study was too small to draw conclusions on this issue. In general, the risk of neurological features due to atlanto-axial instability, spinal canal stenosis or instability at other spinal levels, seemed to be relatively low. However, spinal complications might have been higher if our study had been conducted among a group of adult patients.

Ophthalmological and Hearing Complications

There were both concordant and discordant findings regarding ophthalmological and hearing manifestations between this cohort and previous studies, demonstrating the importance of caution in interpreting genotype–phenotype correlations based on relatively small numbers.

Myopia was found in 45% of the patients, being moderate or severe in more than half. In some instances, we could document the progressive nature of myopia. Retinal detachment developed at a wide range of ages. A more severe ocular phenotype was observed in patients with glycine to aspartic acid and glycine to arginine mutations compared to patients with glycine to serine substitutions. However, we found several adult patients with glycine to non-serine mutations, who did not have eye problems. This observation differed from that of Nishimura et al. who reported ocular abnormalities in all patients with glycine to non-serine substitutions [Nishimura et al., 2005]. Arginine-to-cysteine mutations (p.Arg719Cys; p.Arg989Cys) did not seem to cause ocular problems, which was consistent with previous observations that ocular involvement did not occur in patients with a substitution of an arginine in the Y position [Nishimura et al., 2005; Hoornaert et al., 2010; Zhang et al., 2011]. In our cohort, patients with splicing mutations were at particularly high risk for myopia and retinal

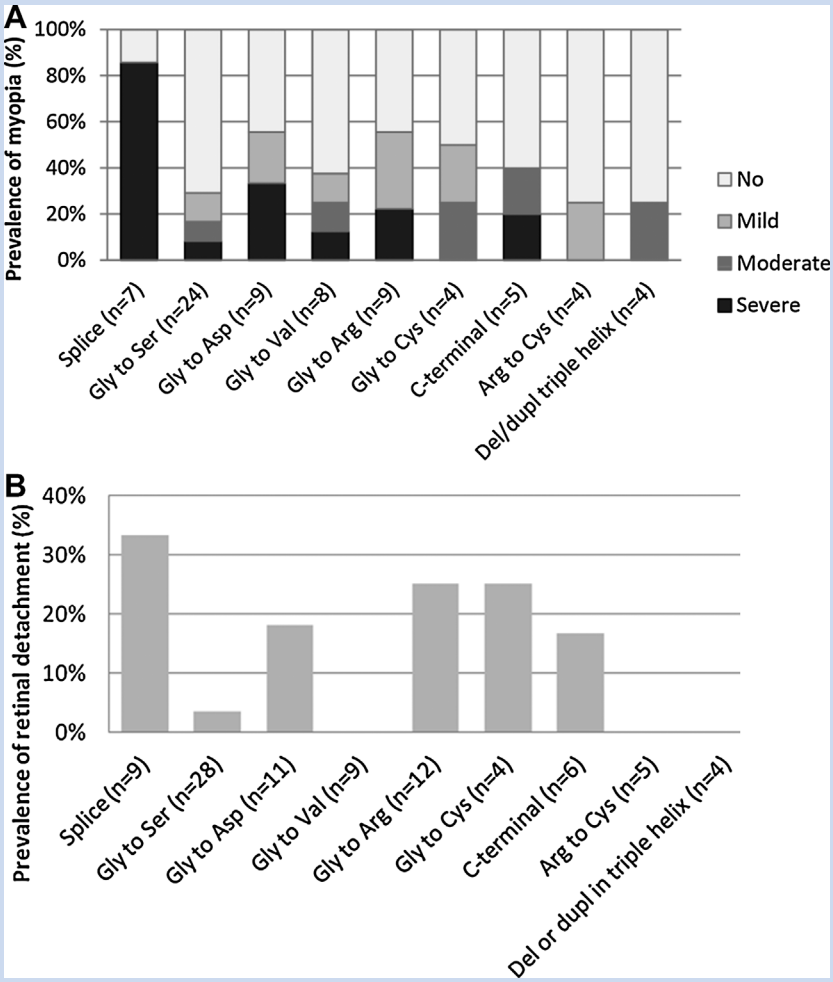

FIG. 10. A: Presence and severity of myopia, according to mutation group, n = number of patients from which information is available. B: Occurrence of retinal detachment, according to mutation group, n = number of patients from which information is available.

detachment. This is consistent with the conclusion of Nishimura et al. and other authors [Winterpacht et al., 1993; Bogaert et al., 1994; Spranger et al., 1997; Wilkin et al., 1999; Yokoyama et al., 2003; Nishimura et al., 2005; Walter et al., 2007].

Hearing loss was detected in all mutation groups, contrasting with the study of Nishimura et al. who concluded that hearing loss was uncommon in their patients with glycine substitutions [Nishimura et al., 2005]. In our cohort, three of the six patients with a C-terminal mutation complained of hearing loss, one of whom required hearing aids. These findings differ from previous studies where hearing impairment was infrequently seen in patients with mutations in the C-terminal propeptide [Zankl et al., 2004; Nishimura et al., 2005]. The high percentage of patients with splicing mutations using hearing aids suggests that, similar to the ophthalmological features, hearing loss is more severe in this group. This is in keeping with previous studies [Winterpacht et al., 1993; Bogaert et al., 1994; Spranger et al., 1997; Wilkin et al., 1999; Yokoyama et al., 2003; Nishimura et al., 2005; Walter et al., 2007].

To investigate why ocular and auditory problems were more frequent and severe in patients with a splicing mutation, we performed RNA studies in two patients (patient 40 and patient 88) and showed that alternatively spliced transcripts with intra-helical deletions are formed (Supplementary Table SIII). Others have reported similar findings and proposed that, as well as accumulating in the endoplasmic reticulum, these isoforms can also become incorporated into type 2 collagen molecules leading to a qualitative defect by looping out of the normal chains [Fernandes et al., 1998; Weis et al., 1998]. The ocular and auditory systems may be more sensitive to this kind of defect.

Follow-Up and Management

Our study highlights several important factors that should be taken into consideration in the management and follow-up of patients with non-lethal type 2 collagen disorders (Table II, Key points for counseling). Close ophthalmological and audiological

TABLE II. Key Points for Counseling and Follow-Up Recommendations for SEDC or a Related Phenotype

Clinical features
- Respiratory problems after birth are reported in 26% (95% CI 17–37)
- A cleft palate and a clubfoot deformity are present in respectively 22% (95% CI 14–32) and 9% (95% CI 4–18) of the neonates
- Motor development is delayed in 34% (95% CI 24–46). Median age at walking is 18 months (IQR 15-23 months)
- Retinal detachment occurred in 12% (95% CI 6–21) of the patients.
- Complaints of hearing loss are reported in 37% of the patients
- In 32 patients where cervical radiographs were available for evaluation, 56% (95% CI 38–74) had odontoid hypoplasia. Based on evaluation of flexion-extension films in 18 patients, 28% (95% CI 10–54), had atlanto-axial instability

Recommendations for follow-up

Ophthalmological	First exam in neonatal period, with follow-up visit at age 6 months, at age 1 year, and thereafter annually until adulthood
Hearing	Otoacoustic emissions (OAE) in neonatal period, or, if not available: auditory brainstem evoked response (ABR). Patient-specific follow-up, but at least until speech development
Osteoarticular	Flexion/extension films of the cervical spine at age 3 years and before surgery. If abnormal, referral to a neurologist and consider additional investigations (MRI). If there is doubt about atlanto-axial stability, repeat flexion-extension films at age 7 years
Multidisciplinary	Annual assessment by an orthopedic surgeon, pediatrician, physiotherapist and rehabilitation specialist

follow-up is required, particularly in patients with a splicing mutation. If the diagnosis is made in the neonatal period, we advise performing an ophthalmological examination in the first weeks of life, as one patient in our study was diagnosed with congenital buphthalmos. Contact sports should be discouraged due to the risks of both retinal detachment and cervical instability and patients (or their parents) should be made aware of neurological warning symptoms suggestive of myelopathy, such as frequent falling, abnormalities of micturition or defecation, or disturbed sensation. When surgery or anesthesia are required, the neck should be handled with caution.

In conclusion, the information provided in our study is a further step toward more evidence-based management and counseling for care providers dealing with a patient affected by a type 2 collagen disorder. Further follow-up studies, preferably carried out in a large group of adult patients, may help to delineate complication profiles specific to individual mutations or mutation groups.

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