Novel mutations of the *PRKAR1A* gene in patients with acrodysostosis


Acrodysostosis is characterized by a peripheral dysostosis that is accompanied by short stature, midface hypoplasia, and developmental delay. Recently, it was shown that heterozygous point mutations in the *PRKAR1A* gene cause acrodysostosis with hormone resistance. By mutational analysis of the *PRKAR1A* gene we detected four different mutations (p.Arg368Stop, p.Ala213Thr, p.Tyr373Cys, and p.Arg335Cys) in four of seven affected patients with acrodysostosis. The combination of clinical results, endocrinological parameters and *in silico* mutation analysis gives evidence to suppose a pathogenic effect of each mutation. This assumption is supported by the *de novo* origin of these mutations. Apart from typical radiological abnormalities of the hand bones, elevated thyroid stimulating hormone and parathyroid hormone values as well as short stature are the most common findings. Less frequent features are characteristic facial dysmorphisms, sensorineural hearing loss and mild intellectual disability. These results lead to the conclusion that mutations of *PKRAR1A* are the major molecular cause for acrodysostosis with endocrinological abnormalities. In addition, in our cohort of 44 patients affected with brachydactyly type E (BDE) we detected only one sequence variant of *PRKAR1A* (p.Asp227Asn) with an unclear effect on protein function. Thus, we conclude that *PRKAR1A* mutations may play no major role in the pathogenesis of BDE.

Conflict of interest

The authors declare no conflict of interest.
Acrodysostosis (OMIM #101800) is a rare form of peripheral dysostosis, which was first described by Maroteaux and Malamut (1) and is associated with brachydactyly, short stature, midface hypoplasia, accelerated bone age and developmental delay (2). Radiological findings include short metacarpals as well as phalangeal bones, cone-shaped epiphyses, decreased interpedicular distances and spinal stenosis (3, 4). Although most cases occur sporadically, autosomal dominant inheritance patterns have been reported (5, 6).

Recently, it has been discovered that mutations in two genes, namely PRKAR1A (protein kinase, cyclic adenosine monophosphate (cAMP)-dependent, regulatory type I, alpha; OMIM *188830) and PDE4D (phosphodiesterase 4D, cAMP-specific; OMIM *600129), which both play a role in the cAMP pathway, cause acrodysostosis with or without hormone resistance (7–9). The PRKAR1A gene codes for the regulatory subunit type I alpha (R1α) of the cAMP-dependent protein kinase A (PKA).

In the absence of cAMP, PKA exists as an inactive tetrameric holoenzyme consisting of two regulatory (R) and two catalytic (C) subunits (10). After binding of cAMP to the R-subunits they dissociate from the C-subunit, which can now start its phosphorylase activity (11).

The detachment of R1α from the C-subunit is mediated by two steps of cAMP binding. First, cAMP has to interact with the nucleotide-binding domain (NBD) B to get access to the NBD-A which, when interacting with cAMP, allows the detachment of the C-subunit from the R1α-subunit (12).

In consequence, PRKAR1A loss-of-function mutations that will decrease the quantity of R1α or interfere with its binding to the C-subunit may cause an increased PKA activity, as it is the case in Carney complex type I (OMIM #160980) patients. This syndrome is characterized by diverse myxomatous tumors as well as other neoplasms, pigmented skin lesions and a hormonal hyperfunction (13, 14).

Mutations of the R1α subunit, which reduce the affinity to cAMP, hamper the dissociation of the R- and C-subunits, and hence decrease PKA activity. It is likely that this mechanism of reduced PKA signaling causes the hormonal and skeletal phenotype of acrodysostosis.

Brachydactyly type E (BDE) is a non-specific anomaly defined by a shortening of the metacarpal and/or metatarsal bones. Acrodysostosis and BDE show overlap in terms of shortened metacarpal bones. BDE is genetically heterogeneous and can be caused by mutations in the PTHLH (parathyroid hormone-like hormone) gene (OMIM #168470) (15). PTHLH mediates its effect by the PTHR (parathyroid hormone receptor), a receptor of the G protein family (16) which activates phospholipase C as well as adenylyl cyclase (17), hence they are involved in a pathway that includes PKA. Because only a minority of BDE cases can be explained by PTHLH mutations or deletions, we screened a cohort of individuals affected by BDE to learn whether mutations in PRKAR1A, an effector downstream in the PTHLH pathway, can also cause BDE.

In this study, we present one recurrent and three previously not described mutations in the PRKAR1A gene in four of seven patients with acrodysostosis and one missense variant of PRKAR1A in an individual with BDE. We discuss the possible pathogenicity of the newly discovered mutations with the help of in silico analysis and data on existing R1α variants. We compare our results to the data of the patients from the literature.

Materials and methods

Patients

The cohort consisted of 7 patients with acrodysostosis and 44 patients with BDE. For inclusion in the group of acrodysostosis, diagnostic criteria were severe brachydactyly of the metacarpal, metatarsal and phalangeal bones in combination with cone-shaped epiphyses, short stature and/or facial dysmorphism such as midface hypoplasia. The BDE group consisted of patients who showed a shortening of at least one metacarpal or metatarsal bone but otherwise did not meet the aforementioned criteria of acrodysostosis. The endocrinological data, radiographs and information on the phenotype of the patients with acrodysostosis were evaluated by one of the coauthors (D. H.). We obtained parental DNA samples from three of four patients with acrodysostosis where we found a mutation. All of the BDE patients were sporadic cases and had previously been screened for mutations in the PTHLH and GNAS genes. No mutations were found.

Informed consent

Written informed consent was given by the patients or the legal guardian for genetic testing and publication of images.

Mutational analysis

For the amplification of the coding exons 2–11 (including the flanking intronic sequences) of PRKAR1A primers were designed with the Primer3 (v. 0.4.0) software (http://frodo.wi.mit.edu/) (Table 1). The polymerase chain reaction (PCR) was performed with AmpliTaq DNA Polymerase (Applied Biosystems, Warrington, GB). Each PCR consisted of 2.0 μl × 10 PCR buffer (Applied Biosystems), 0.2 μl dNTP mix (20 mM each), 0.5 μl primer (10 pmol/μl) and 1 μl of template DNA (30–60 ng/μl). The reaction for exon 6 included 2 μl dimethyl sulfoxide (DMSO) (Merck, Darmstadt, Germany). Every batch was filled with H2O up to 20 μl total volume. We used the following PCR cycling conditions: 94°C (240 s); 3 cycles of 94°C (30 s), 61°C (45 s), 72°C (60 s); 3 cycles of 94°C (30 s), 59°C (45 s), 72°C (60 s); 3 cycles of 94°C (30 s), 57°C (45 s), 72°C (60 s); 31 cycles of 94°C (30 s), 55°C (45 s), 72°C (60 s) and 72°C for 10 min.
Novel mutations of PRKAR1A

Table 1. List of primers

<table>
<thead>
<tr>
<th>Localization</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Size of fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>5′-AAATCCTGTGAGTCATTGTC-3′</td>
<td>5′-TTGGAACCCCTTTATATGCG-3′</td>
<td>527</td>
</tr>
<tr>
<td>Exon 3</td>
<td>5′-GAACATGAGGTCACCAGGCTT-3′</td>
<td>5′-CCCAAGAATTGTACAGGATG-3′</td>
<td>366</td>
</tr>
<tr>
<td>Exon 4</td>
<td>5′-TATTACGCTGCCGCTGTTGAG-3′</td>
<td>5′-TTCCTTTCTCTGTAATTTTCA-3′</td>
<td>488</td>
</tr>
<tr>
<td>Exon 5</td>
<td>5′-TAAAGGTGTGATCCCAAATGGT-3′</td>
<td>5′-CCAATACAAAGTTGTCGCTATC-3′</td>
<td>415</td>
</tr>
<tr>
<td>Exon 6</td>
<td>5′-CCCTGAAAGATTGTTGATTTGTG-3′</td>
<td>5′-GCTCGAAAGCGATCAATACA-3′</td>
<td>244</td>
</tr>
<tr>
<td>Exon 7</td>
<td>5′-TCGTCAGAAATCACCTATCTTCTC-3′</td>
<td>5′-AGCTGCGGTATAATGCAAGT-3′</td>
<td>497</td>
</tr>
<tr>
<td>Exon 8</td>
<td>5′-TCCATAGCATTGTTGGTGATA-3′</td>
<td>5′-TCCTAAAGCTTCCATCAATTCT-3′</td>
<td>199</td>
</tr>
<tr>
<td>Exon 9</td>
<td>5′-GCGATGGCTTATTTGTTGAAA-3′</td>
<td>5′-TTAGCCACCTTCTTCTCTTCT-3′</td>
<td>318</td>
</tr>
<tr>
<td>Exon 10</td>
<td>5′-TCTGACCTCCCTTTAAGCACT-3′</td>
<td>5′-GCAAGATGGGTACACGCTAA-3′</td>
<td>565</td>
</tr>
<tr>
<td>Exon 11</td>
<td>5′-GTGCAGCTGCTTTAAGGAAATGTT-3′</td>
<td>5′-CAGACAGGAAGCTGCGATG-3′</td>
<td>357</td>
</tr>
</tbody>
</table>

Following an enzymatic purification using the Sanger method using 0.5 μl BigDye Terminator v3.1 (Applied Biosystems), 0.75 μl BigDye Terminator v1.1/3.1 buffer (×5) (Applied Biosystems), 0.5 μl of the respective forward or reverse primer, respectively, and filled up to 5 μl with H2O.

Sequencing was performed using the 3730 DNA Analyzer (Beckman Coulter, Krefeld, Germany). Evaluation of the sequences was conducted with the SeqMAN TM II software (DNASTAR, Madison, WI). An independent sequencing reaction was performed to confirm each mutation. To rule out single-nucleotide polymorphisms we screened DNA samples of 200 Caucasian controls for mutations in the affected exons.

In silico analysis

For an in silico analysis we evaluated the mutations with MUTATIONTASTER (http://www.mutationtaster.org/) (18) and POLYPHEN-2 software (http://genetics.bwh.harvard.edu/pph2/) (19). To examine the degree of conservation of the affected amino acids we retrieved the protein sequence of 10 species (chimpanzee, macaque, cow, mouse, platyfish, chicken, zebrafish, dog, opossum, and orang-utan) via the Ensembl Genome Browser (http://www.ensembl.org/index.html) and conducted a multiple sequence alignment with CLUSTALW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (20).

Results

In our cohort of seven patients with acrodysostosis we identified four mutations in the PRKAR1A gene (Fig. 1). In a single patient with acrodysostosis we observed the previously described p.Arg368Stop mutation. Besides this nonsense mutation we observed only missense mutations (Table 2). We could confirm the de novo origin in three of these four mutations. In the fourth case, we were unable to obtain parental DNA samples. Furthermore, a variant of PKR1A was detected in one individual with BDE. In addition, sequencing analysis of PRKAR1A in 200 control individuals of Caucasian origin did not reveal any of the sequence changes observed in our patients or any alterations that would lead to changes of the affected amino acids.

MUTATIONTASTER predicted a 99.99% probability for each missense mutation to have a pathogenic effect. POLYPHEN classified four mutations as ‘probably damaging’ but predicted a benign effect of the p.Asp227Asn mutation found in the individual with BDE.

In the conservation analysis, we observed that the amino acids altered by the c. 637G>T mutations were conserved over 10 species, while the Tyr373 of the c.1118A>G mutation was conserved in all species except the opossum.

Three of the mutations found are localized in the NBD-B (p.Tyr373Cys, p.Arg368Stop, and p.Arg335Cys). Two sequence changes were observed in the NBD-A (p.Asp227Asn and p.Ala213Thr).

Clinical data

Patients with acrodysostosis analyzed in this study had small hands and feet with short metacarpals, metatarsals and phalangeal bones (Figs 2 and 3). Cone-shaped epiphyses were observed in all patients reported in this study (Fig. 2, Table 3). Not all of these patients exhibited short stature (body height ranged from −1.1 standard deviation (SD) to −3.3 SD). One patient presented a normal height (>−2 SD) but displayed midface hypoplasia. Patient 7 with a height of −3.3 SD was...
Table 2. PRKAR1A mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Mutation</th>
<th>Localization</th>
<th>De novo verification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acrodysostosis</td>
<td>c.637G&gt;A (p.Ala213Thr)</td>
<td>PBC of the NBD-A</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Acrodysostosis</td>
<td>c.1118A&gt;G (p.Tyr373Cys)</td>
<td>NBD-B</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Acrodysostosis</td>
<td>–</td>
<td>–</td>
<td>n.a.</td>
</tr>
<tr>
<td>4</td>
<td>Acrodysostosis</td>
<td>c.1102C&gt;T (p.Arg368Stop)</td>
<td>NBD-B</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Acrodysostosis</td>
<td>–</td>
<td>–</td>
<td>n.a.</td>
</tr>
<tr>
<td>6</td>
<td>Acrodysostosis</td>
<td>–</td>
<td>–</td>
<td>n.a.</td>
</tr>
<tr>
<td>7</td>
<td>Acrodysostosis</td>
<td>c.943C&gt;T (p.Arg315Cys)</td>
<td>PBC of the NBD-B</td>
<td>No parental DNA</td>
</tr>
<tr>
<td>8</td>
<td>BDE</td>
<td>c.679G&gt;A (p.Asp227Asn)</td>
<td>NBD-A</td>
<td>No parental DNA</td>
</tr>
</tbody>
</table>

BDE, brachydactyly type E; n.a., not analyzed; NBD, nucleotide-binding domain; PBC, phosphate-binding cassette.

of Vietnamese origin, whereas the other patients were of Caucasian origin.

We only had detailed clinical information of five individuals affected with acrodysostosis; of these two showed facial dysmorphisms, i.e. flat nasal bridge or hypertelorism (Table 3). In addition, patient 7 had a sensorineural hearing loss. Mild developmental delay was present in patient 1.

Our endocrinological data is not complete on every patient analyzed in this study. However, parathyroid hormone (PTH) value was elevated for the individuals affected with acrodysostosis in whom we detected a mutation (Table 3). Childhood patients had PTH values higher than the standard for adults (1.5–6 pmol/l or 11–67 pg/ml). The thyroid stimulating hormone (TSH) values were elevated for all individuals in this group with the exception of patient 2 who was on thyroxine medication (100 μg/day). This leads to the conclusion that the TSH values must have been elevated at some point in his past. The only patient who had reported a normal PTH level was patient 5, in whom we could not identify a PRKAR1A mutation; no TSH value was available for her (Table 3).

Other endocrinological findings included an increased follicle stimulating hormone level in the male patient 2 (9.1 IU/l, n = 1–8 IU/l) which is consistent with a resistance to hypophysial releasing hormones. Interestingly, patient 4 who carried the p.Arg368Stop mutation showed an elevated basal human growth hormone (HGH) level (13.45 ng/ml, n = 0.30–12.10 ng/ml) and she did not present with short stature (height of 139.3 cm at the age of 11.5 years = −1.1 SD).

In the radiographs of her hands, patient 8 with BDE only showed a short fourth metacarpal, as well as a short distal phalanx of the thumb but no cone-shaped epiphyses (Fig. 2). She presented with short stature (−2.7 SD) but no dysmorphic facial features were documented. Endocrinological testing did not reveal the characteristic elevated PTH and TSH levels.

Discussion

We identified one recurrent and three novel mutations of the PRKAR1A gene in four of seven patients with acrodysostosis. To date, 25 patients with acrodysostosis and a mutation in PRKAR1A have been reported (7–9, 21, 22). Seventeen of them (including one of ours) carry the most common mutation c.1102C>T (p.Arg368Stop). All of the other detected mutations, except one (Gln372Stop) (22), are missense mutations. Most of the known mutations alter the NBD-B, which has been described as the ‘gatekeeper’ for cAMP-dependent activation of the PKA (12), and therefore, mutations in this domain are predicted to reduce the PKA response to cAMP to a greater degree than

Fig. 2. Hand radiographs of patients in whom a PRKAR1A mutation was detected. (a) patient 1, (b) patient 2, (c) patient 4, (d) patient 7, and (e) patient 8. Patients 1–7 show a shortening of the majority of the metacarpal and phalangeal bones as well as cone-shaped epiphyses mostly of the proximal phalanges. The X-ray of patient 8 affected with brachydactyly type E differs with only showing a short fourth metacarpal and distal phalanx of the thumb. No cone-shaped epiphyses are observed.
Novel mutations of *PRKARIA*

alterations in the NBD-A. The NBD-A is an uncommon location for mutations. Only two of the patients with acrodysostosis showed a mutation in this part of the protein (the patient described by Nagasaki et al. (21) and our patient 1). Also the c.679G>A mutation observed in the BDE patient is located in the NBD-A. The mutation found in patient 4 (p.Arg368Stop) has been described several times (7, 9, 22) and there is proof that it reduces cAMP-dependent PKA activation (7).

Concerning the mutations p.Arg335Cys and p.Tyr373Cys, amino acid exchanges at the same positions have been detected in other patients with acrodysostosis (8, 9, 22). In addition, Herberg et al. (12) created a porcine RIα which was mutated at Arg333 (Arg333Lys) corresponding to Arg335 in humans and which leads to higher levels of cAMP needed for holoenzyme activation. A similar finding was reported for the p.Tyr373Cys mutation where Bubis et al. (23) created a porcine Tyr371Phe RIα variant (corresponding to the human Tyr373) which reduced cAMP affinity to the NBD-B and hampered holoenzyme activation.

Interestingly, a mutation of the Alanine at position 213 of the RIα has been described in patients with Carney complex type 1 (24). We found a mutation at the same amino acid position in patient 1 (p.Ala213Thr), located in the NBD-A. Greene et al. (24) observed a reduced cAMP affinity to the RIα subunit but the R-subunit itself had a lower affinity to the C-subunit. This leads to an increased amount of free, catalytic active C-subunit and thus, an increased PKA activity. This corresponds with the findings by Kim et al. (25). They showed that the highly conserved amino acids of the phosphate-binding cassette of the NBD-A, on the one hand, play a significant role in cAMP binding to RIα via interactions with the cAMP phosphate group. On the other hand, they are important for the binding of RIα itself to the C-subunit, mainly by hydrophobic interaction. Thus, this mutation which leads to the Carney complex may hinder the association of the RIα- and C-subunit, and therefore may result in a PKA hyperfunction.

Patient 1 did not show any pigmentary changes consistent with the Carney complex (only one café au lait spot on the lower back). Neither myxomas nor any other neoplasias have been documented in this patient. Her endocrinological findings, especially the elevated TSH levels in combination with a normal free thyroxine (fT4), could be interpreted as a hormone resistance, in contrast to the hormonal hyperfunction which is seen in Carney complex patients. In conclusion, the clinical findings allow the diagnosis of acrodysostosis in this individual. Furthermore, mild developmental delay found in this patient has been reported several times in connection with acrodysostosis (2, 26).

For the contradicting result that a mutation of the same codon can lead to either enhanced or reduced PKA signaling, we suggest that the p.Ala213Thr mutation
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (year), sex</th>
<th>Measurements</th>
<th>Shortened metacarpalia, metatarsalia and phalangea, cone-shaped epiphyses</th>
<th>Facial dysmorphisms</th>
<th>Laboratory findings</th>
<th>Psychomotor development</th>
<th>Additional data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15, female</td>
<td>Height 143 cm (−3.3 SD) Weight and head circumference normal</td>
<td>+</td>
<td>−</td>
<td>Elevated TSH (6.21 mIU/l); elevated PTH (91 pg/ml)</td>
<td>Mild developmental delay</td>
<td>Coarse hair, short neck, café au lait spot on the back, narrow interpedicular distances</td>
</tr>
<tr>
<td>2</td>
<td>31, male</td>
<td>Height 152.5 cm (−3.3 SD) Head circumference 58 cm (+0.81 SD)</td>
<td>+</td>
<td>−</td>
<td>Elevated PTH (8.8 pmol/l) and FSH (9.1 U/l); normal TSH (1.49 mIU/l) on thyroxine medication (100 μg/day)</td>
<td>Normal</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>nd, female</td>
<td>nd</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>4</td>
<td>11.5, female</td>
<td>Height 139.3 cm (−1.1 SD) Weight 55.5 kg (+1.46) Head circumference 56.7 cm (+2.7 SD)</td>
<td>+</td>
<td>Hypoplastic midface, short philtrum, prognathia, oligodontia</td>
<td>Elevated TSH (7.41 mIU/l) and HGH (13.45 ng/ml); no PTH available</td>
<td>Normal</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>11, female</td>
<td>Height 128 cm (−2.3 SD)</td>
<td>+</td>
<td>−</td>
<td>Standard values for calcitonin and PTH; TSH not reported</td>
<td>Normal</td>
<td>Broad and flat thorax</td>
</tr>
<tr>
<td>6</td>
<td>nd, female</td>
<td>nd</td>
<td>+</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>7</td>
<td>31, female</td>
<td>Height 135 cm (−3.3 SD) Head circumference 52 cm (−1.8 SD)</td>
<td>+</td>
<td>Nasal hypoplasia</td>
<td>nd</td>
<td>Normal</td>
<td>Sensorineural hearing loss, short neck, disproportionate short stature</td>
</tr>
<tr>
<td>8</td>
<td>44, female</td>
<td>Weight 38 kg (−3.6 SD) Height 150 cm (−2.7 SD) Only shortened fourth metacarpals</td>
<td>−</td>
<td>−</td>
<td>Standard values for PTH and TSH</td>
<td>nd</td>
<td>–</td>
</tr>
</tbody>
</table>

FSH, follicle stimulating hormone; HGH, human growth hormone; PTH, parathyroid hormone; TSH, thyroid stimulating hormone; not documented (nd).

*TSH standard: 0.3–2.5 mIU/l, PTH standard: 12–72 ng/l or 1.56.0 pmol/l, HGH standard: 0.30–12.10 ng/ml, FSH standard: 1–8 IU/l (males).
Novel mutations of PRKAR1A

In summary, the clinical phenotype caused by the PRKAR1A mutations detected in our cohort shows variability. The radiographic findings of the hands, i.e. short metacarpals and phalangeal bones as well as cone-shaped epiphyses, are present in every patient. The endocrinological anomalies are found in all individuals with PRKAR1A mutations in whom we obtained this data. Typical facial anomalies may be associated with PRKAR1A mutations (Fig. 4), but are not present in all our patients with acrodysostosis carrying a PRKAR1A mutation. One patient (patient 4) did not exhibit short stature at the age of 11.5 years but displayed characteristic facial features. Hearing loss and developmental delays are rare findings among these patients.

Patient 8, who carries the p.Asp227Asn mutation, was the only individual in the cohort of BDE patients in whom we detected a sequence variant. Unfortunately her parents were deceased and, therefore not available for genetic testing. Consequently, we cannot prove whether this sequence change was de novo. We could not identify it in a control group consisting of 200 individuals. The MUTATIONTASTER predicted a 99.99% probability for the pathogenicity of this mutation, but POLYPHEN-2 predicted a ‘benign’ effect of the amino acid exchange. The crystal structure of PRKAR1A (Bos taurus) and its binding to either the dissociated cAMP-bound conformation (B-form) or the cAMP-free holoenzyme conformation (H-form) have been studied by crystallography (27). Therefore, we investigated the arguable mutation p.Asp227Asn using the entries 1NE6 and 1NE4 in the Protein Data Bank (www.pdb.org) for rendering in Swiss-PdbViewer. Mutation of Asp227 to Asn showed no effect on binding capacity of PRKAR1A to cAMP. This result emphasizes the hypothesis that this PRKAR1A mutation is not responsible for the BDE phenotype. However, whether the sequence change is at least in part responsible for the phenotype of patient 8 can only be investigated by further functional testing.

Analyzes of further 43 individuals with BDE gave normal results. Therefore, it seems unlikely that PRKAR1A mutations are a major cause of BDE. We suggest that disturbed R1α activity would lead to wider effects than isolated shortness of metacarpals/metatarsals.

We found novel PRKAR1A mutations in addition to the one previously described in patients with acrodysostosis (7–9, 21, 22). Three patients did not harbor a mutation of PRKAR1A and these individuals should be then screened for PDE4D mutations. Three out of four of our acrodysostosis patients affected by a PRKAR1A mutation had abnormal endocrinological findings. In the fourth patient, no endocrinological data was obtainable. With only rare findings of altered hormonal function in patients with PDE4D mutations being described in the literature (8, 9, 22), we conclude that in patients with endocrinological anomalies such as elevated PTH or TSH first a sequencing of PRKAR1A is indicated and without such findings the analysis of PDE4D should be prioritized.

Fig. 4. Variability of the facial phenotype seen in patients with acrodysostosis and PRKAR1A mutations. Patient 1 (a, b) and patient 4 (c, d). While patient 4 displays facial dysmormisms like midface hypoplasia, small nose with a flat nasal bridge, and short philtrum which are characteristic of acrodysostosis, patient 1 shows none of these findings. Patient 4 further shows very thin lips, which is also seen in patient 1.
Muhn et al.

Acknowledgement

We thank the patients and their families for participating.

References

25. Wu J, Jones JM, Nguyen-Huu X, Ten Eyck LF, Taylor SS. Crystal structures of R1alpha subunit of cyclic adenosine 5’-monophosphate (cAMP)-dependent protein kinase complexed with (Rp)-adenosine 3’’,5’’-cyclic monophosphothioate and (Sp)-adenosine 3’’,5’’-cyclic monophosphothioate, the phosphothioate analogues of cAMP. Biochemistry 2004: 43: 6620–6629.