Intranasal insulin to improve the developmental delay in children with 22q13 deletion syndrome: an exploratory clinical trial

Heinrich Schmidt, Werner Kern, Renate Giese, Manfred Hallschmid and Angelika Enders

doi:10.1136/jmg.2008.062141

Updated information and services can be found at:
http://jmg.bmj.com/cgi/content/abstract/jmg.2008.062141v1

Notes

Online First contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

To order reprints of this article go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to Journal of Medical Genetics go to:
http://journals.bmj.com/subscriptions/
Intranasal insulin to improve the developmental delay in children with 22q13 deletion syndrome: an exploratory clinical trial

Intranasal insulin in 22q13 deletion syndrome

Heinrich Schmidt MD1, Werner Kern MD2, Renate Giese3, Manfred Hallschmid PhD4, Angelika Enders MD3

Department of Paediatric Endocrinology and Medical Genetics1, Department of Paediatric Neurology3
Dr. von Hauner Children’s Hospital
University of Munich
Lindwurmstr. 4
80337 Munich/Germany

Departments of Internal Medicine I2 and Neuroendocrinology4
University of Lübeck2
Ratzeburger Alle 160
23538 Lübeck/Germany

Corresponding author:
Heinrich Schmidt MD
Dr. von Hauner Children’s Hospital
University of Munich
Lindwurmstr. 4
80337 Munich
Germany
Tel: 0049-89-5160-2811
Fax:0049-89-5160-4192
E-mail: heinrich.schmidt@med.uni-muenchen.de

Abbreviations: ARSA, arylsulfatase A; ProSAP2, proline-rich synapse-associated protein 2; Shank3, SH3 and multiple ankyrin repeat domains 3.
Abstract

**Background/Aims.** 22q13 deletion syndrome (Phelan-McDermid syndrome) is characterized by a global developmental delay, the absence or delay of speech, generalized hypotonia, autistic behaviour and characteristic phenotypic features. Intranasal insulin has been shown to improve declarative memory in healthy adult subjects and patients with Alzheimer’s disease. We assessed if intranasal insulin is also able to improve the developmental delay in children with 22q13 deletion syndrome.

**Methods.** We performed exploratory clinical trials in 6 children with 22q13 deletion syndrome who received intranasal insulin over a period of one year. Short-term (during the first 6 weeks) and long-term effects (after 12 month of treatment) on motor skills and cognitive functions as well as autonomous functions, speech and communication, emotional state, social behaviour, behaviour disorders, independence in daily living and education were assessed.

**Results.** The children showed marked short-term improvements with regard to gross- and fine motor activities, cognitive functions and educational level. Positive long-term effects were observed for fine- and gross motor activities, nonverbal communication, cognitive functions and autonomy. Possible side effects were observed in one patient who displayed changes in balance, extreme sensitivity to touch and general loss of interest. One patient complained of casual nose bleeding.

**Conclusions.** We conclude that long-term administration of intranasal insulin may benefit motor development, cognitive functions and spontaneous activity in children with 22q13 deletion syndrome.

**Key words:** Intranasal insulin, cognitive functions, 22q13 deletion syndrome, Phelan-McDermid syndrome, *ProSAP2/Shank3* gene haploinsufficiency.
The 22q13 deletion syndrome (Phelan-McDermid syndrome) that was first described by Watt and coworkers in 1985 [1] is characterised by a global developmental delay, generalized hypotonia, the inability to reach the milestones for various physical and mental activities, severe expressive speech delay or even loss of verbal skills, normal or accelerated growth and minor physical features [2-4]. Of major interest in this microdeletion syndrome is the general developmental impairment, in particular of cognition and memory functions. It is assumed that these features result from haploinsufficiency of the ProSAP2/Shank3 gene that triggers the production of postsynaptic scaffolding proteins. Consequently, the development of dendritic spines is impaired [5].

Besides its involvement in glucose metabolism, insulin also acts as a neuropeptide in the central nervous system, modulating plastic neuronal mechanisms assumed to be involved in memory processing. When administered intranasally, insulin reaches the cerebrospinal fluid and alters brain functions without relevant absorption into the blood stream [6]. Intranasal insulin administration improves declarative memory in healthy subjects and patients with Alzheimer’s disease [7-12]. Assuming that intranasal insulin may be able to at least partially compensate for the cognitive deficits caused by ProSAP/Shank3 haploinsufficiency, we performed exploratory clinical trials in six children with 22q13 deletion syndrome.

**Study design**

Six children with 22q13 deletion syndrome (patients’ characteristics are given below) received intranasal insulin treatment for 12 months. For intranasal administration, insulin (40 IU/ml; Actrapid, Novo Nordisk, Mainz, Germany) was diluted with 0.9% saline solution to a concentration of 20 IU/ml so that each 0.1 ml puff with the nasal atomizer (Aero Pump, Hochheim, Germany) contained a dose of 2 IU insulin. Subjects received one dose of 2 IU insulin per day during the first 3 days according to the standard subcutaneous insulin therapy in children with type 1 diabetes mellitus. In three-day intervals, administration was increased gradually, until the final dosage of about 0.5-1.5 IU/kg/d (TID) was reached. Blood glucose was controlled with a portable glucometer 30 minutes after each administration during the initial treatment period, i.e., until the maximal treatment dosage was reached. **Outset fasting blood glucose levels in all patients were between 4.2 and 5.0 mmol/l.**

Neurodevelopmental examinations were performed before treatment and during treatment around one year later. Electroencephalography was performed before the start of treatment. Parents were asked to observe and record (on video tape) new skills, changes in autonomous functions, changes in movement, cognitive abilities, speech development and communication skills, emotional state, social contact, changes in behaviour and independence in daily living. After six weeks and after one year of treatment, they were asked to fill in a questionnaire assessing short-term (first 6 weeks) and long-term (12 months) treatment effects on a scale ranging from -10 (most extreme degree of aggravation) to + 10 (extremely positive development), with 0 indicating no changes [13]. **The German version [14] has been extended to include additional specific items and a more detailed rating scale. It is an established tool used in the daily routine to assess developmental changes in mentally disabled children that contains 9 sections of questions regarding the developmental status (Table 1).** Ratings were compared with clinical observations made by the examiner and with observations of psychologists, physiotherapists and occupational therapists made during routine examinations. All 6 patients underwent regular examinations by the same examiner. Anthropometric data (height, weight, head circumference) were collected and blood levels of glucose, cortisol and insulin antibodies were determined after 6 and 12 months of treatment. Written informed consent was obtained from each individual family.
Patients and outcome

**Patient 1:** Karyotype: 46,XY, r(22).ish r(22)(p13q13.1)(TUPLE1+, bcr+, ARSA-,Tel22q-); two year old male patient, with marked cognitive developmental delay, absence of expressive speech, autistic behavior, minor but generalized muscular hypotonia with uncoordinated ataxic movements (walking at 15 months), restlessness, marked sleep disturbance, persistent screaming, mouthing and chewing, normal growth, large fleshy hands and feet, deep set eyes, pointed chin, dolicocephaly, dysplastic finger- and toenails. Auxologic data at the start of treatment (age 3 years): 13.7 kg (10-25%), 96.5 cm (25-50%); MRI: arachnoidal cyst and large cisterna magna. Medication: levomepromazin, phenobarbital, pipamperon. Therapy: physiotherapy, occupational therapy.

After a few weeks of treatment (maximal dose of intranasal insulin: 20 IU/d), obvious changes were observed with regard to sweating, sleeping without interruption, emotional balance, motor functions (e.g. moving with improved postural control, steering a tricycle, drawing a line with a pencil), concentration over longer time periods (e.g., looking at books, remembering animals after visiting the zoo). The child understood more words, showed empathy and could help when getting dressed. The child also stopped to show stereotype behavior. After 12 months of treatment, the improvement continued, especially regarding sleep rhythm, motor skills, attention span, as well as expressing emotions. No progress in speech development was recorded (Table 2). Side effects: none reported.

**Patient 2:** Karyotype: 46,XX, del(22)(q13).ish del(22)(q13.3q13.3)(ARSA-), 9 months old girl at the time of diagnosis. At the age of ten days a large ovarian cyst had been removed. She was growing too fast during the first two years of life and had recurrent upper airway infections. At the age of 16 months she exhibited a global developmental delay. Phenotypic features: flat middle face, high forehead, deep-set eyes, ptosis, a bulbous nose, dysplastic nails, finger pads (Figures 1 and 2). Auxologic data at the start of treatment (age 16 months): 12 kg (97%), 86cm (>97%); MRI: a thinned-out corpus callosum; no medication. Therapy: physiotherapy.

The short-term effects of insulin (maximal dose 17.5 IU/d) were normalisation of obstipation and body temperature, higher agility, progress in gross and fine motor functions (e.g., reaching across the midline of the body, coordination of complex movements), more perseverance in activities, longer concentration spans. However, as a consequence of increased mobility, also more risk-taking behavior was observed. Long-term effects were observed for autonomous functions (e.g., stable body temperature, less infections, uncomplicated eating behaviour, chewing), gross and fine motor functions (e.g., more body tension, grasping with more precision), and cognitive performance (e.g., longer attention spans). Progress could be observed in speech understanding, nonverbal communication (e.g., pointing with her finger), and emotional regulation. The girl rarely showed stereotype behavior. She actively took part in dressing and undressing and could anticipate dangerous situations quite well (Table 2.). Side effects: none reported.

**Patient 3:** Karyotype: 46, XX, del(22)(q13.33), a 3 year old girl at the time of diagnosis. Although she showed hypotonia during the first two years, she could sit and walk without assistance quite early. Vocalization started at the age of two. The patient was skiing and swimming at the age of 8.5 years. She spoke two-word sentences. She was restless with short spans of attention, as well as showing aggressive and autistic behaviour. Phenotype: deep-set eyes, dysplastic flat finger nails. Therapy: physiotherapy, speech therapy, occupational therapy. Auxologic data at the start of treatment (age: 8.5 years):28 kg (50-75%), 123cm (3-10%), head circumference: 52 cm (50-75%); therapy: physiotherapy, speech therapy, occupational therapy.

Short-term effects (maximal dose 12 IU/d): the patient showed less motor activity and was walking with an unstable gait. She appeared to be very introverted and not interested in
her surroundings. She did not like to be touched and her behavior was much more difficult to control. Therefore, treatment was stopped after 5 weeks. Subsequent effects (assessed after treatment has ended): Especially during the subsequent 3 months, the girl exhibited marked progress in gross and fine motor abilities (e.g., she learned to swim) as well as in her cognitive abilities (e.g., she could play with her dolls over a period of 20 minutes). She showed significant progress in verbal communication (e.g. using more words and sentences; expressing wishes by using words, e.g., “May I go outside?”) and social behavior (e.g., she was interested in other children and wanted to play with them). Education and behavior control in every-day life were much easier.

**Patient 4**: Karyotype: 46, XX, del(22)(q13.32 mos), a 6.5 year old girl at the time of diagnosis. At the age of 7 months she was able to sit, at the age of 15 months she was able to walk. She could also ride a bicycle and climb up trees. At six years of age, she had a vocabulary of 40-50 words and she could communicate in short sentences. She became dyspractic and showed a marked loss of speech. At the age of nine she exhibited autistic-like, self-aggressive behaviour, pain intolerance, as well as restlessness. She could read and calculate with three figure numbers. Phenotypic features: deep-set eyes, a rounded tip of the nose, a prominent chin. Auxologic data at the start of treatment (age 9.5 years): 33 kg (75-90%), 130.8 cm (25-50%); therapy: lamotrigin, speech therapy and facilitated communication.

Short-term effects (maximal dose 14 IU/d): The girl was in a more calm and balanced mood. She showed better gross and fine motor abilities (increased motor activity with more precision in her movements, e.g., in threading pearls), prolonged attention spans (e.g., she typed up to two pages with assistance) and was more alert. She seemed to be more competent in situations of daily living and showed less self-aggressive behavior, was more compliant to educational interventions and took part in social activities. Positive long-term effects were assessed especially with regard to motor skills, autonomy and activities of daily life (e.g., slicing bread, using the remote control of the TV set, unloading the dishwasher). In general, the girl’s attention span became longer and she showed a remarkable progress in her playing behavior. She showed more interest in other children and strong positive emotional reactions to other people (e.g., with longer periods of eye contact, showing happiness and sadness, comforting others). She began to imitate speech and to communicate with special devices. In everyday living she was more ready to compromise, could cope better with frustration and showed more flexibility. Side effects: none reported.

**Patient 5**: Karyotype: 46, XY, del (22)(q13.3), a 20 month old boy at the time of diagnosis. He was sitting at 24 months, walking at 36 months, showed muscular hypotonia, was growing too fast during the first three years of life, developed hydronephrosis due to obstructive uropathy. His speech abilities at 10 months consisted of double syllables and stagnated afterwards. At the age of five he showed autistic-like behaviour, restlessness, short attention spans, self-aggressive behaviour, and no verbal communication. Phenotypic features: a rounded tip of the nose, puffy eyelids, persistent embryonal finger pads, flat and hypoplasic finger-nails. Auxologic data at the start of treatment (age 5.5 years): 21.2 kg (90%), 113.5cm (75-90%); therapy: physiotherapy.

Minor short-term effects (maximal dose 18 IU/d) like increased motor activity and better general attention were observed. Positive long-term effects could be observed in gross motor coordination abilities (e.g., climbing stairs without assistance), and concentration (e.g., being able to play longer). The boy showed more interest in his surroundings (e.g., in listening to rhythms and songs). He reacted to speech and used nonverbal communication devices. He showed jealousy as well as joy to see his siblings again after being separated from them for some time (Table 2). Side effects: occasional nose bleedings.

**Patient 6**: Karyotype: 46, XX, del (22)(q13.3).ish del(22)(q13.3)(ARSA-), a 12 month old girl at the time of diagnosis. She showed muscular hypotonia, was sitting at the age of 12
months, could not walk without assistance at two years. Her growth range was between 50 and 75%, and she showed normal sweating. She had an obstructive uropathy on the right side and a mild aortal stenosis. At 26 months of age, she could only walk with holding on to someone’s hand. She showed autistic-like behaviour with no speech development and restlessness. She did not use pincer grip and did not notice objects when they disappeared.

Phenotypic features: a rounded tip of the nose, deep-set eyes and squint, flat and hypoplastic finger-nails. Auxologic data at the start of treatment (aged 26 months): 10.6 kg (3-10%), 89cm (50-75%); therapy: physiotherapy.

Short-term effects (maximal dose 8 IU/d): The girl suddenly began to like eating and did not protest against being fed. She showed progress in gross and fine motor abilities (e.g., she was able to point at pictures, she began to walk and could stand up without holding on). Her attention span was significantly increased. She showed more interest in her social surroundings, began to imitate gestures and to use non-verbal communication devices. She gained much more emotional stability and flexibility. Side effects: none reported. This patient dropped out during treatment so that potential long-term effects could not be observed.

Effects of insulin treatment are summarized in Table 2 and averages are depicted in Figure 3. In all patients, the short- and long-term effects observed and documented by the parents were comparable with the observations of the examiner and the clinical staff. Notably, all patients whose children showed improvements wished the treatment to be continued. No treatment effects on blood values including glucose, HBA1c, cortisol and insulin antibodies were observed after 12 months of therapy. Auxologic data remained normal.

Discussion

In an exploratory clinical trial, six children suffering from 22q13 deletion syndrome were intranasally administered insulin for up to one year. Observations made by the children’s parents, by the experimenter and by routine clinical staff not involved in the experiments (i.e., observers blinded to insulin treatment) jointly indicate a beneficial effect of intranasal insulin on the cognitive and also motor development of the patients, suggesting that intranasal insulin might be a valuable tool to ameliorate the condition of patients with 22q13 deletion syndrome.

Due to the small number of patients, their different chromosomal abnormalities (ring chromosome, mosaicism, large vs. smaller deletions) as well as to the variability of their age and physical/cognitive impairments, our results can only be taken as a first indication of the beneficial effects of intranasal insulin in patients suffering from 22q13 deletion syndrome. Short-term effects of intranasal insulin were assessed in all 6 patients, whereas long-term effects of continued insulin administration were determined only in 4 patients. Here, the first positive changes were registered after one week (4-6 IU insulin/d) of treatment, with particular improvement of restlessness and prolongation of attention span. After six weeks, in four of the six patients, significant progress could primarily be seen in control and coordination of gross as well as in fine motor functions, attention span and control of behaviour in every day life. These changes were corroborated by similar observations made by non-involved therapists. After one year of treatment, the four children under observation exhibited progress primarily in motor functions (e.g. strength, new acquired functions), in understanding speech, in applying communication devices as well as in prolonged attention spans, improved hand functioning and more autonomy in every day life.

Patient 3, the least impaired patient, was the only one who showed unexpected negative symptoms concerning mood, movement and tactile sensitiveness in the first weeks of treatment. These changes disappeared after the premature end of treatment and were followed by remarkable improvements during the subsequent 3 months. Because patients with 22q13 deletion syndrome often show regression of skills and subsequent regain, it is difficult to
attribute this course of events to the insulin treatment although insulin might have contributed to initially enhanced and, thus, overstraining perception. One patient (Patient 5) only showed minor beneficial short-term effects and less pronounced long-term effects. Apart from occasional nose bleeding in one patient, no other side effects could be registered, excluding in particular hypoglycaemia due to intranasal insulin.

The mechanisms behind our observations cannot be derived from our data. The ProSAP2/Shank3 gene is preferentially expressed in cortex, cerebellum and hippocampus. It encodes a protein involved in the stability of the postsynaptic density (PSD) of excitatory neurons (a highly specialized submembraneous network of proteins). Therefore, haploinsufficiency of the ProSAP2/Shank3 gene as found in patients with 22q13 deletion syndrome might impair the development of dendritic spines and even cause degradation of synapses, resulting in the observed impairments in cognition and gross- and fine motor functions [5].

Intranasal administration effectively delivers neuropeptides to the CNS, bypassing the blood-brain barrier and avoiding systemic side effects. Using this route of administration, a direct effect on central nervous signalling pathways is possible [6;15;16]. Intranasal administration of insulin has been shown to influence hippocampus-dependent declarative memory function in healthy volunteers [7-10]. Furthermore, intranasal insulin improves cognition and mood in patients with Alzheimer’s disease [11;12]. Insulin may be expected to increase central nervous glucose uptake, thereby improving neuronal function, which is particularly relevant in patients with cognitive impairments [17]. Synergistically, insulin may enhance synaptic plasticity via glutamatergic/GABAergic receptors and via phosphoinositol 3-kinase dependent mechanisms although these processes probably develop with some delay [18]. It has been demonstrated that IRS p53, an insulin receptor tyrosine kinase substrate, may improve PSD by interacting with ProSAP/Shank and may contribute to the morphological reorganization of spines and synapses after insulin receptor activation [19]. Intranasal insulin may stimulate this process also in case of reduced availability of ProSAP/Shank proteins by increasing the expression of the local dendritic scaffolding protein PSD 95, thus partially compensating for the reduced availability of ProSAP/Shank proteins and inducing cognitive improvements. Previously, intranasal insulin was shown to be more effective in patients with cognitive impairments who do not carry the APOE-ε4 allele, a genetic risk factor for Alzheimer’s Disease [20]. Also, women appear to benefit from the cognitive effects of intranasal insulin to a greater extent than men [9]. The APOE-ε4 status of our patients is not known and the small sample size prevents any conclusions on differential gender effects. It would likewise be of interest if the reaction to intranasal insulin is affected by deletion size that has been previously shown to correlate with some features of developmental retardation [21]. Clearly, future studies will have to address the question how these factors may influence insulin effects in children with developmental delay.

This is the first report on intranasal insulin treatment in children, in particular in patients with 22q13 deletion syndrome. As the natural development in those children is unknown and the exploratory nature of our experiments prevented the inclusion of a placebo group, only tentative conclusions can be drawn from our results. Further and more systematic studies will be necessary to elucidate if the beneficial effects observed in our study will also be obtained in greater samples of patients, with a particular view on possible side effects of the compound. Enhanced dose regimens might even lead to more pronounced developmental improvements. The clinically astonishing short-term changes observed here indicate an improving effect of intranasal insulin treatment, suggesting that insulin administered in parallel with physiotherapy, speech therapy and occupational therapy may support learning processes in patients with 22q13 deletion syndrome.
Acknowledgments
Mister V., father of patient V.G. (patient 1), made these clinical trials possible by intensive
literature research efforts, incited by the tireless intention to improve his son’s quality of life.
Aero Pump GmbH (Hochheim/Germany) generously provided us with precision nasal air
pump. We thank Barbara Berner for linguistic advice.
The Corresponding Author has the right to grant on behalf of all authors and does grant on
behalf of all authors, an exclusive licence (or non exclusive for government employees) on a
worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article to
be published in Journal of Medical Genetics and any other BMJPGL products to exploit all
subsidiary rights, as set out in our licence
The parents of Patient 2 have given their consent for the photographs of their child to appear
in the Journal of Medical Genetics and associated publications.
References


<table>
<thead>
<tr>
<th>Section</th>
<th>Categories and items</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Autonomous functions</td>
<td>1. Sweating/body temperature/digestion/defecation (e.g., sweats more, not so hard any more, body temperature better regulated)</td>
</tr>
<tr>
<td></td>
<td>2. Sleeping behaviour (e.g., sleeps peacefully, sleeps less/more, can stay awake longer during daytime)</td>
</tr>
<tr>
<td></td>
<td>3. Eating behaviour (e.g., shows more appetite, eats more, can be fed longer, with less problems, can feed himself...)</td>
</tr>
<tr>
<td>2. Motor skills</td>
<td>4. Activity level (e.g., moves more/less than before)</td>
</tr>
<tr>
<td></td>
<td>5. Hand movements (e.g., grips, manipulates, points better than before…)</td>
</tr>
<tr>
<td></td>
<td>6. Gross motor functions (e.g., has made progress, more strength, is more stable…)</td>
</tr>
<tr>
<td>3. Cognitive abilities</td>
<td>7. Attention span (e.g., when listening, looking at books, playing)</td>
</tr>
<tr>
<td></td>
<td>8. Interest and motivation in new situations and activities (e.g., explores more …)</td>
</tr>
<tr>
<td></td>
<td>9. Memory functions (e.g., seems to remember sequences better, understands and remembers rules of playing…)</td>
</tr>
<tr>
<td>4. Speech and Communication</td>
<td>10. Understanding speech (e.g., understands speech better, is more attentive in listening…)</td>
</tr>
<tr>
<td></td>
<td>11. Speech and vocalization (e.g., uses new sounds, new syllables, new words…)</td>
</tr>
<tr>
<td></td>
<td>12. Use of nonverbal/alternative ways of communication (e.g., looks longer at someone, points, uses gestures in order to express needs…)</td>
</tr>
<tr>
<td>5. Emotional state</td>
<td>13. Emotional stability (e.g., agitation, self injury, crying, screaming, shouting, calms down quicker…)</td>
</tr>
<tr>
<td></td>
<td>14. Child expresses feelings like, e.g., joy, sadness, anger, interest</td>
</tr>
<tr>
<td></td>
<td>15. Child reacts to someone else’s feelings, cries or laughs with others</td>
</tr>
<tr>
<td>7. Behavior disorders</td>
<td>17. Stereotype movements (e.g., repetitive movements with hands or feet, reaction to stress), preferences in playing behaviour (e.g., needs special smells or surfaces….)</td>
</tr>
<tr>
<td>8. Independence in daily living</td>
<td>18. Changes in daily activities (e.g., feeding, dressing, choosing, deciding, using the toilet…)</td>
</tr>
</tbody>
</table>
9. Education

19. Education in activities of daily living, e.g., child shows more obedient behaviour, accepts compromises more easily, tolerates changes better

20. Child anticipates dangers/ needs control
Table 2. Effects of 6 weeks (6 wk) and 12 month (12 mo) of intranasal insulin administration as assessed with a questionnaire on developmental progress [13].

<table>
<thead>
<tr>
<th>Patient</th>
<th>Questions</th>
<th>1 male 3 years</th>
<th>2 female 16 months</th>
<th>3 female 8.5 years</th>
<th>4 female 9.5 years</th>
<th>5 male 5.5 years</th>
<th>6 female 26 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6wk</td>
<td>12mo</td>
<td>6wk</td>
<td>12mo</td>
<td>6wk</td>
<td>12mo</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-1</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>-7</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>-3</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>-8</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>-1</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>7</td>
<td>-5</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>-5</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>-3</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>-9</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>5</td>
<td>5</td>
<td>-7</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Age at the time treatment onset is indicated. Note that treatment was stopped after 5 weeks in Patient 3 but subsequent long-term effects were assessed; no long-term data are available for Patient 6.
Figure Legends

Figure 1. Characteristic facial phenotype (Patient 2). This patient’s parents have given their consent for this photograph of their child to appear in the Journal of Medical Genetics and associated publications.

Figure 2. Dysplastic toe nails (Patient 2). This patient’s parents have given their consent for this photograph of their child to appear in the Journal of Medical Genetics and associated publications.

Figure 3. Effects of 6 weeks (n=6; white bars) and 12 months (n=4; grey bars) of intranasal insulin administration as assessed with a questionnaire on developmental progress [13]. Sum scores (mean +/- SEM) are indicated for 9 sections of questions with a range from –10 (most extreme degree of aggravation) to +10 (extremely positive development), with 0 indicating no changes.