

## Clinical, anthropometric, radiological and molecular characteristics of Egyptian achondroplasia patients

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### ABSTRACT

**Background:** Achondroplasia is the most common form of non lethal skeletal dysplasia. It is a fully penetrant autosomal dominant disorder and the majority of cases are sporadic resulting from de novo mutations associated with advanced paternal age. The phenotype of achondroplasia is related to disturbance in endochondral bone formation due to mutations in the fibroblast growth factor receptor-3 (FGFR3) gene.

**Aim of the Work:** Evaluation of the cardinal phenotypic features in achondroplasia, the body physique using anthropometric measurements, the characteristic radiological signs in the patients as a main tool for diagnosis and detection of the most common mutations in achondroplasia patients in the studied sample.

**Subjects and Methods:** From 42 cases referred to us as achondroplasia, we selected 20 cases where clinical manifestations were consistent with achondroplasia. Cases were subjected to full clinical examination, detailed anthropometric measurements, whole body skeletal survey and molecular studies of the most common mutations of the FGFR3 gene using PCR amplification technique.

**Results:** Nineteen cases were sporadic (95%) and one case had an affected father (5%). A paternal age above 35 years at the time of child's birth was present in 7 cases (35%). Paternal exposure to occupational heat was noted in 6 cases (30%) and parental exposure to chemicals in 3 cases (15%). All cases showed typical clinical and radiological manifestations of achondroplasia. Anthropometric measurements quantitatively confirmed the body physique in the studied cases. G380R common mutations of the FGFR3 gene were detected in 15/18 cases (83%) with the G to A transition at nucleotide 1138 in 14 cases (77%). Agenesis of corpus callosum, not previously reported in association with achondroplasia, was present in the only case with the G-C transversion mutation at nucleotide 1138 (5%).

**Conclusions:** Awareness of the cardinal features of achondroplasia, proper anthropometric measurements and detailed skeletal survey are the key for accurate diagnosis, genetic counseling and avoidance of over diagnosis. The majority of studied Egyptian achondroplasia patients have the same common mutation that has been most often defined in patients with achondroplasia from other countries.

**Key Words:**

Achondroplasia, fibroblast growth factor receptor3, skeletal dysplasia, paternal heat exposure.

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**INTRODUCTION**

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Achondroplasia (OMIM: 10800)<sup>1</sup> is a genetic disorder evident at birth. It occurs in all races and in both sexes. It is the most common non lethal type of skeletal dysplasia and the most common type of short-limb dwarfism. Its depiction in ancient Egyptian art makes it one of the oldest recorded birth defects<sup>2</sup>. Achondroplasia affects more than 250,000 individuals worldwide<sup>3</sup>. It is a fully penetrant autosomal dominant disorder with an incidence rate ranging between 1/15,000 and 1/40,000 live births<sup>4</sup>. The majority of cases are sporadic resulting from de novo mutation associated with advanced paternal age<sup>5</sup>. The phenotype of achondroplasia is related to a disturbance in endochondral bone formation due to mutation in the fibroblast growth factor receptor-3 (FGFR3) gene and consequently, affected individuals exhibit characteristic clinical and radiological features.<sup>6</sup>

Phenotypically, achondroplasia is considered a short-limb dwarfing condition with the proximal segments more severely affected than the distal segments<sup>7</sup>. The hands are short and broad with fingers exhibiting a three-pronged (trident) appearance at birth due to an inability to fully oppose the third and fourth digits. Other characteristic features include, a large head, frontal bossing, flattening of the nasal bridge, midface hypoplasia and relatively prominent mandible. Macrocephaly is due to ventricular enlargement in many cases; however, intracranial pressure is not elevated significantly. The chest

is flattened and the abdomen protrudes with thoraco-lumbar kyphosis, with age exaggerated lumbar lordosis becomes more prominent. Sleep apnea, respiratory difficulties and otitis media are common complications. Spinal deformities are potentially disabling problems as the short thickened pedicle and reduced inter-pedicular distance reduce the size of the spinal canal, both antero-posteriorly and transversely.<sup>8</sup>

A skeletal survey is of utmost importance to confirm the diagnosis of achondroplasia. In children caudal narrowing of the inter-pedicle distance rather than the normal caudal widening and a notch like sacro-iliac groove are typical features in addition to the other radiological findings characteristic of the limbs, vertebrae and skull.<sup>9</sup>

Once the gene for achondroplasia was assigned to 4p16.3 by linkage analysis, causative mutations were identified by the candidate gene approach<sup>10</sup>. Achondroplasia is caused by missense mutation in the fibroblast growth factor receptor-3 gene (FGFR3), which is located at 4p16.3. Two mutations in the FGFR3 gene, a G-to-A transition at nucleotide 1138 and a G-to-C transversion at the same nucleotide, both resulting in G380R amino acid substitutions, cause over 99% of cases with achondroplasia.<sup>11</sup>

The purpose of our study was to evaluate the cardinal phenotypic features in achondroplasia, the body physique us-

ing anthropometric measurements, the characteristic radiological signs in the patients as a main tool for diagnosis and to detect the most common mutations in achondroplasia patients in our sample.

## **SUBJECTS AND METHODS**

This study included 20 cases with achondroplasia. Cases were selected from 42 patients referred as achondroplasia to the Limb Malformations and Skeletal Dysplasia Clinic, Medical Services Unit at the National Research Centre (NRC), Cairo, Egypt in the last two years. Cases were selected based upon the presence of the typical characteristic stigmata of achondroplasia. The study included 11 females and 9 males; their mean age at presentation was 3.42 years, ranging from 9 months to 9 years. A full explanation of the study has been provided to the studied cases and their parents and a written consent has been obtained.

For each case the followings were conducted:

- Detailed clinical examination with special emphasis on dysmorphic features, skeletal system, limb segments and examination of different body systems. Clinical examination was carried out for parents and sibs.
- Anthropometric measurements including height (Ht), weight (Wt), head circumference (HC) and sitting height. Body mass index (BMI) was calculated for each case ( $BMI = Wt \text{ in kilograms}/Ht^2 \text{ in meters}^2$ ). Measurements were compared to age and sex matched normal Egyptian control<sup>12,13</sup>. Statistical analysis including standard deviation scores (SDS) were calculated. Upper segment (US)/ lower segment (LS) ratio was calculated. Total limb length, arm length, forearm length and hand length were measured and compared to normal standards<sup>14</sup>. Absolute measurements for Ht and HC were plotted on achondroplasia curves.<sup>15</sup>
- A skeletal survey including radiographs of skull, spine, pelvis, short and long bones. Neuroimaging of the brain including CT or MRI scan.
- Molecular studies were conducted on 18 cases, who provided their consent for blood withdrawal, using the following steps:
  - DNA has been extracted from fresh peripheral blood leucocytes by standard salting out protocol.<sup>16</sup>
  - PCR amplification across the transmembrane domain, which contains the common mutations G1138A and G1138C of the FGFR3 gene. PCR
- Three - generation pedigree construction including consanguinity, similar conditions and other affected members in the family.
- Complete history including parents, occupation, pregnancy and delivery histories, exposure to drug intake, fever, trauma, irradiation, or any maternal chronic illness. Parental ages at birth of the child, family history and developmental milestones as well as the occurrence of complications as respiratory problems, otitis media and history of orthopedic complications.

amplification was performed in a 50  $\mu$ l PCR reaction containing 50 ng of genomic DNA as a template, 100  $\mu$ M of each dNTPs, 10 pmol of each primer, 1 unit Taq polymerase and its buffer (Fermentase).

- o Restriction endonuclease analysis was the method used to detect the presence or absence of the common mutation. SfcI was the restriction enzyme applied to detect the G to A transition at nucleotide 1138; the enzyme has been incubated with the completed PCR product at 37°C. MspI restriction enzyme was used to detect the G to C transversion at the same nucleotide position 1138 of FGFR3.
- o Digested samples were loaded on 3% ethidium stained agarose gel, electrophoresed and visualized on UV-transilluminator.

## RESULTS

This study included 20 cases (11 females & 9 males) and one father with the characteristic features of achondroplasia. Parental consanguinity was noticed in only 2 cases (10%). The paternal ages at the time of the child's birth ranged from 22 to 41 years. A paternal age above 35 years was present in 7 cases (35%) while a maternal age of 35 was present in one case only (5%). Ninety five percent of cases were sporadic; only one case (5%) had a father with achondroplasia (Figure. 1).

History of prolonged paternal exposure to heat as a result of occupation was noted in 6 cases (30%), three of the exposed fathers were at the mid twenties and 3 were at the early thirties. Parental exposure to chemical agents was pres-

ent in 3 cases (15%); a mother had Daflon for treatment of varicose veins at the 5<sup>th</sup> month of pregnancy, a father had lipidol injection 6 months before conception and another was working in the petrol and chemical industry for more than 10 years.



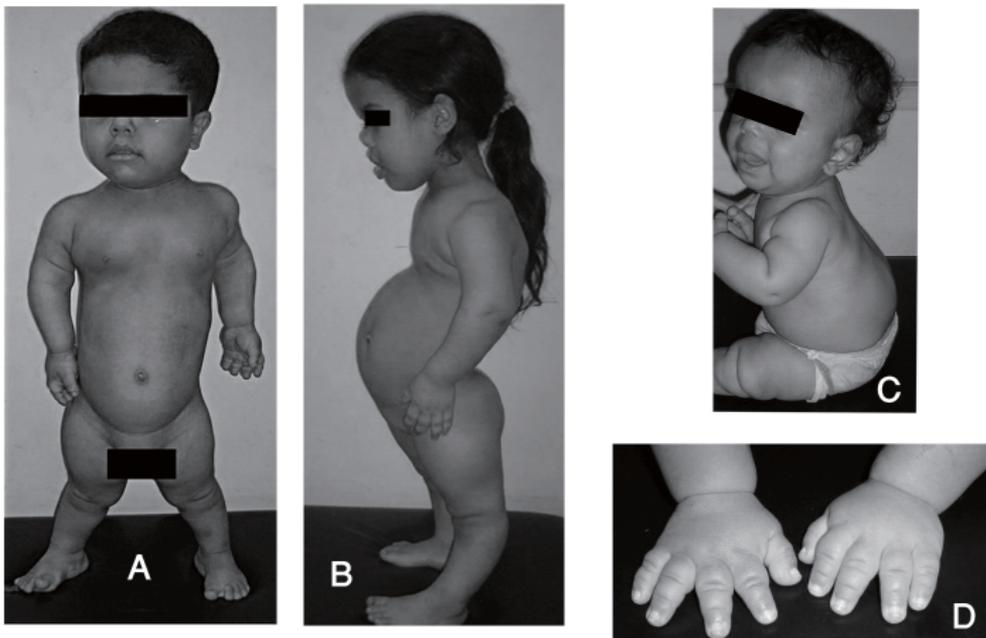
**Fig. 1:** A father (A) and his son (B) with the characteristic features of achondroplasia.

### Results of clinical findings:

All studied cases had the characteristic features of achondroplasia as shown in (Figures. 1, 2). The characteristic facial phenotype in the form of frontal bossing, mid face hypoplasia, depressed nasal bridge and short neck was present in all cases. Examination of upper and lower limbs in the studied cases revealed that all cases had apparent rhizomelic shortening, limited elbow extension and generalized joint laxity in addition to brachydactyly and trident

hand configuration. Bowing of legs or genu varum was noticed in all except 5 cases (75%). Many cases had multiple skin creases over the limbs (Michelin tire creases). Examination of the trunk revealed spinal deformities in the form of lumbar kyphosis in 5 cases (25%) while exaggerated lumbar lordosis was present in 75% of cases. Protuberant abdomen was noticed in all cases. Heart, chest and abdomen were clinically free

in all cases. Upper respiratory tract infection, recurrent snoring, sleep apnea and otitis media were common complications in most cases. Neurological examination revealed hypotonia in 70% of cases. Brisk reflexes were present in 6 cases (30%) with dilated ventricles and cortical atrophic changes evident by brain neuroimaging. Motor developmental milestones were delayed in 50% of cases.



**Fig. 2:** The characteristic clinical features of achondroplasia (A), exaggerated lumbar lordosis and short limbs (B), kyphosis (C), brachydactyly and trident hand configuration (D).

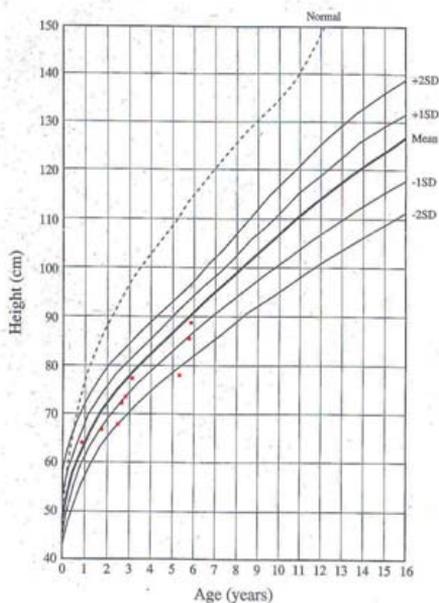
### Results of Anthropometric measurements:

Using available Egyptian standards<sup>12,13</sup>, height was below -2.0 SD in 95% of cases with a mean SDS of  $-4.06 \pm 2.73$ . Only one case who was 9 months old had normal height of -0.76 SD. It was noted that short stature becomes more apparent with age. Mean SDS for sitting height was  $-1.06 \pm 1.7$ . Sitting height was below -2.0 SD in only 5 cases (25%). US/LS ratio was above the 97<sup>th</sup>

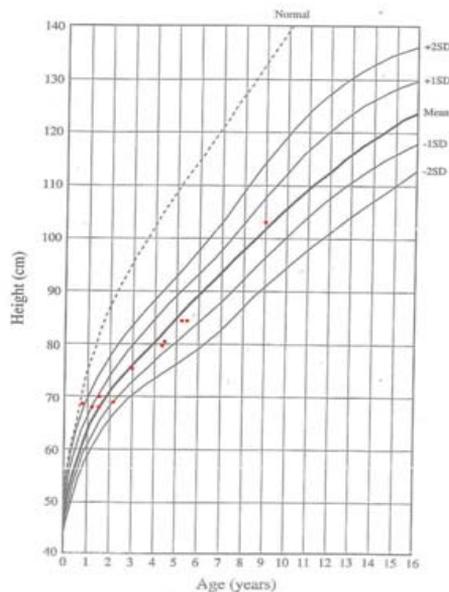
centile in all cases denoting short limbs. Mean SDS for weight was  $-1.41 \pm 1.7$ , only 3 cases showed a SDS below -2.0 SD (15%). Using BMI as an estimate of obesity showed that 12 cases were relatively obese (60%) with a mean SDS of  $2.07 \pm 0.98$  while the rest of cases had normal scores. Macrocephaly (HC above 2.0 SD) was present in 11 cases (55%) with a mean SDS of  $2.06 \pm 1.97$ . Measurements of total limb length, arm, forearm and hand were all below the

3<sup>rd</sup> centile for age compared to normal standards<sup>14</sup>, with more affection of the arm length. All cases were within the normal range using the achondroplasia

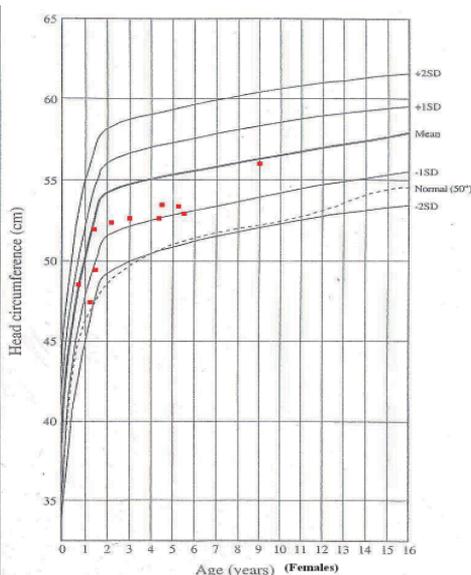
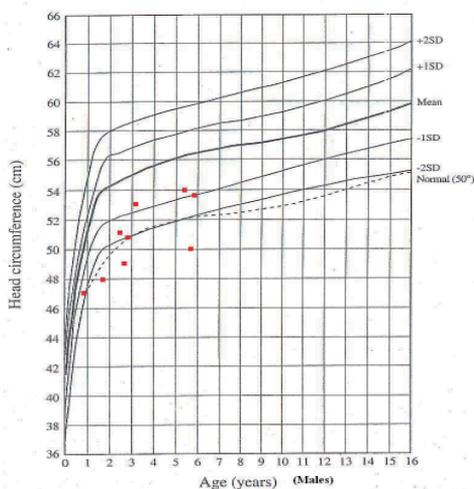
curves<sup>15</sup> for Ht and HC except one case with height just below -2.0 SD and 3 males who had a HC less than -2.0 SD (Figures. 3, 4, 5).



**Fig. 3:** Absolute height measurements of the studied male cases plotted on achondroplasia height chart for males.<sup>15</sup>



**Fig. 4:** Absolute height measurements of the studied female cases plotted on achondroplasia height chart for females.<sup>15</sup>



**Fig. 5:** Absolute head circumference measurements of the studied cases plotted on achondroplasia head circumference charts for both males & females.<sup>15</sup>

**Results of radiological findings:**

Examples of the characteristic radiological features in the studied cases of achondroplasia are shown in (Figure. 6).

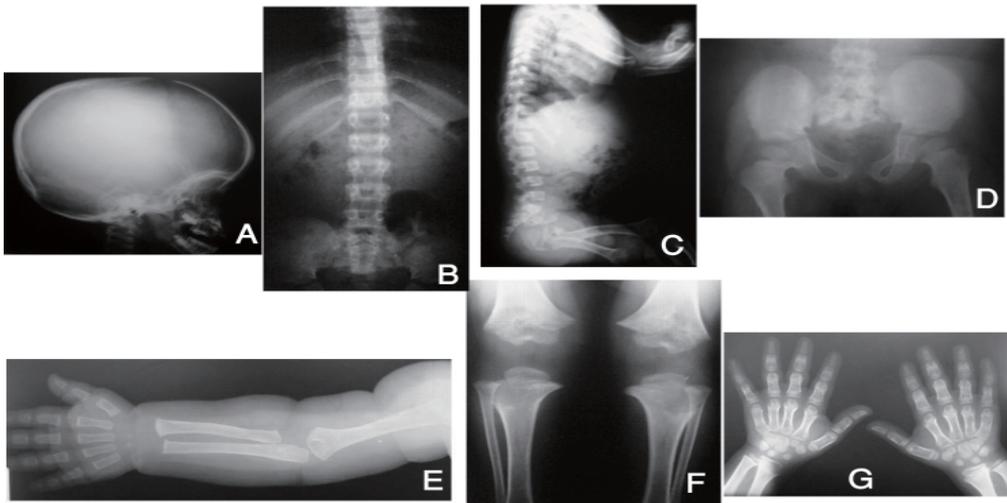
Frontal bossing, relative small skull base and narrow foramen magnum were common radiological findings in the lateral view of skull X-ray.

Anteroposterior (AP) view of the spine revealed caudal narrowing of the interpediculate distance in all cases. Lateral view of the spine revealed shortening of the pedicles with significant posterior scalloping that became more apparent

with age. Exaggerated lumbar lordosis was noticed in most cases (75%).

The pelvis had small square iliac wings, small short sacrosciatic notch, flat horizontal acetabular roof and short femoral neck with elongated trochanters.

Long bones were short and thick with metaphyseal flaring. The humerus was markedly shortened. The distal femoral physes had an inverted-V shaped configuration best noted with age. The fibula was typically longer than the tibia. The hands were broad with short metacarpals and phalanges and a trident configuration.



**Fig. 6:** Examples of the characteristic radiological features of achondroplasia in the studied cases:

**A** - Plain X ray, lateral view of the skull showing frontal bossing, thick calvaria and relatively small skull base.

**B** - Plain X ray, AP view of spine showing caudal narrowing of interpediculate distance.

**C** - Plain X ray, lateral view of vertebrae showing platyspondyly with posterior scalloping and exaggerated lumbar lordosis.

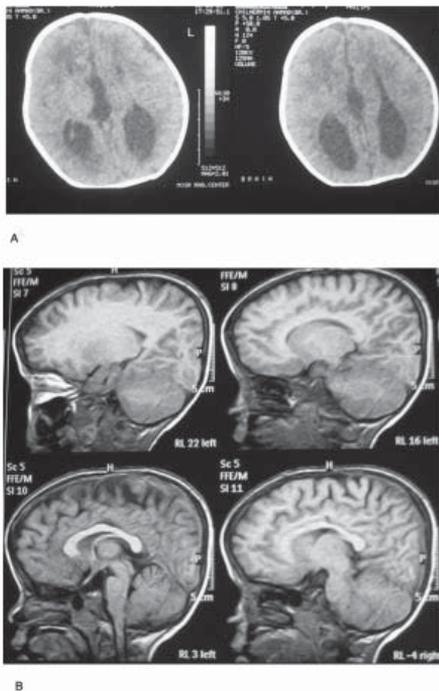
**D** - Plain X ray, AP view of the pelvis showing square iliac wings, flat acetabular roof and narrow sacrosciatic notch.

**E** - Plain X ray of long bones of right upper limb showing short broad long bones mainly in the humerus.

**F** - Plain X ray of long bones of lower limbs showing short broad long bones, metaphyseal flaring of the distal femoral ends taking the form of inverted-V (chevron) shaped metaphyseal notch.

**G** - Plain X ray of both hands showing brachydactyly & trident configuration.

Neuroimaging of the brain revealed abnormal findings in 8 cases (40.0 %). Dilated ventricles and cortical atrophic changes were present in 3 cases (15%). Cortical atrophy was noted in 2 cases (10%) while dilated ventricles only were seen in 1 case (5%). CT scan brain of one case showed agenesis of corpus callosum (Figure. 7 A). MRI of craniocervical region in another case revealed narrow foramen magnum without spinal cord compression (Figure. 7 B).



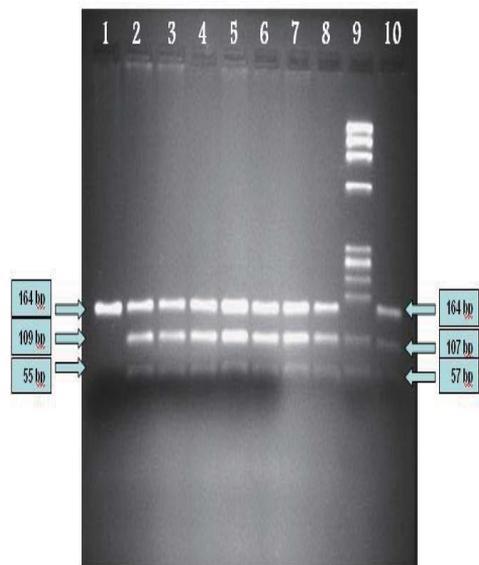
**Fig. 7:** CT scan brain of achondroplasia case with the G to C transversion at nucleotide 1138 of FGFR3 showing: colpocephaly, anterior displacement of 4<sup>th</sup> ventricle & dilated 3<sup>rd</sup> ventricle denoting agenesis of corpus callosum (A).

Sagittal section in MRI of craniocervical region in an achondroplasia case showing narrow foramen magnum without spinal cord compression (B).

**Results of Molecular study:**

The G380R common mutations were detected in 15 patients out of the 18 ascertained cases on clinical and radiological bases (83%). The observed mutation in 14 of the studied cases (77%) was the G to A transition at nucleotide 1138, located in the transmembrane domain of FGFR3 as shown in (Figure. 8). The detected G to A point mutation creates a Scl I enzyme produced two fragments of 109 bp and 55 bp.

On the other hand digestion of the same 164bp PCR fragment with MspI endonuclease (indicating G to C transversion) produced two fragments of 107bp and 57bp. This MspI restriction site was evident in only one patient (5%) (Figure. 8, lane 10). The two common mutations were not detected in three patients (16%), who were consistent with the clinical and radiological criteria of achondroplasia.



**Fig. 8:** FGFR-3 mutations in some of the studied cases with achondroplasia.

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## DISCUSSION

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In our study, all cases were sporadic except one case with autosomal dominant inheritance. Seven cases were associated with advanced paternal age denoting dominant mutations, a finding previously confirmed by Stoll et al.<sup>17</sup> who noticed that de novo gene mutations were associated with advanced paternal age, often defined as over 35 years.

Prolonged heat exposure because of the father's occupation was noted in 6 of the studied cases starting at the age of twenties, a finding that was not specifically reported with achondroplasia but of significant importance as exposure to heat is known to increase the exposure of spermatozoa to mutagenic metabolites. Tarin et al.<sup>18</sup> suggested that heat exposure in the period before conception could theoretically increase de novo mutations or alter the epigenetic modification of imprinted genes, particularly because late spermatids and mature spermatozoa do not have repair enzymes.

Parental exposure to chemical agents in this study was present in 3 cases. Some of the most common exposures that have been studied for increased risk of achondroplasia, but for which no association had been found included: chemotherapy<sup>19</sup>, anticonvulsant drugs<sup>20</sup>, antihistaminics<sup>21</sup>, corticosteroids, calcium channel blockers<sup>22</sup> and thalidomide.<sup>23</sup>

Diagnosis of achondroplasia is based upon clinical findings and confirmed by radiological and molecular studies. All included cases had the typical facial features described in achondroplasia. Although, hyperextensibility of knee, wrist and interphalangeal joints was

noted, full extension and rotation of the elbow was restricted in most cases. Kitoh and associates<sup>24</sup> found limited elbow extension in 68% of their studied cases with achondroplasia. In our study thoracolumbar kyphosis was present in only 5 cases with ages ranging from 9 months to 18 months. Cases above this age had exaggerated lumbar lordosis due to improvement of hypotonia with age. Motor milestones of development were delayed in 50% of our cases due to associated early hypotonia.

In our study all cases were disproportionately short with short limbs mainly proximal and relatively long narrow trunk. Quantitatively confirmed by anthropometric measurements, it was noted that short stature becomes more distinctive with age. US/LS ratio was above the 97<sup>th</sup> centile in all cases indicating that disproportionate short stature was caused primarily as a result of short limbs. Although total upper limb length was below normal in all our studied cases, arm lengths were the mostly affected thus, quantitatively supporting the rhizomelic shortening in achondroplasia. All the clinical and anthropometric data in our cases were consistent with the findings reported by Horton et al.<sup>3</sup>

Although the clinical features of achondroplasia are so distinctive and can be easily identified at birth in most cases, Nicoletti et al.<sup>25</sup> said that about 20% of affected individuals are not recognized at birth. This was evident in case 1 as short stature was not present at the age of 9 months. Achondroplasia curves should be used whenever possible for affected cases. In our study the few cases lying below -2.0 SD on achondroplasia curves can be attributed to the patient's genetic potential, possibility of associated growth hormone deficiency and the use of standards from other

countries due to the unavailability of achondroplasia curves for Egyptians.

Skeletal survey was carried out for all the studied cases to explore the characteristic radiological features of achondroplasia. Our results were consistent with the radiological criteria for diagnosis of achondroplasia reported by Spranger et al.<sup>26</sup>

Although clinical manifestations and radiological investigations are crucial for the diagnosis of achondroplasia, definitive diagnosis and prenatal confirmation in high risk pregnancies are carried out by molecular analysis. In the present study 14 out of 18 cases had the G-A transition and 1 case had the G-to-C transversion at nucleotide 1138 of the FGFR3 gene. Agenesis of corpus callosum was noticed in our case with the G-C transversion mutation. To our knowledge this finding has not been described before in the medical literature neither with achondroplasia in general or with this mutation in particular.

The common mutations detected in our cases were in the heterozygous state, no homozygous gene mutation was identified in our sample of achondroplasia patients. Homozygosity in achondroplasia gene results in a severe lethal skeletal dysplasia with progressive hydrocephalus, brain abnormalities and respiratory failure due to narrow chest and pulmonary hypoplasia. Survival beyond infancy is rare.<sup>27</sup>

Molecular results of common mutations at nucleotide 1138 of the FGFR3 gene of our achondroplasia patients coincide with other studies concluding that the majority of Egyptian achondroplasia patients had the same mutation that has been most often defined in patients with

achondroplasia from other countries. To our knowledge, the only available molecular study on Egyptian patients with achondroplasia was carried out by Abdel-Aleem et al.<sup>28</sup>. The authors detected G380R common mutation with the G to A transition at nucleotide 1138 in 8 out of 11 cases ascertained on clinical basis as achondroplasia. Same results were found in all of the 16 studied Sweden patients with achondroplasia<sup>29</sup>, ten Chinese achondroplastic patients<sup>30</sup>, eleven of 12 Turkish patients<sup>31</sup> and three Thai patients.<sup>5</sup>

The two common mutations were not detected in three of the studied patients who had the clinical and radiological criteria of achondroplasia. This suggests the existence of other less frequent mutations in the FGFR3 gene. Further molecular studies for rare mutations in achondroplasia were not available for us. Reports of achondroplasia cases with mutations other than the common G380R alterations were described by Ikegawa et al.<sup>32</sup>, Superti-Furga et al.<sup>33</sup>, Nishimura et al.<sup>34</sup> and Prinos et al.<sup>35</sup>

Care should be taken when considering the diagnosis of achondroplasia as it must be confirmed by radiological studies showing the definite findings of achondroplasia and confirmed by molecular study if possible. In this study less than 50% of referred cases as achondroplasia (20 out of 42 referred cases) were consistent with the clinical and radiological manifestations of achondroplasia and were included in this study.

Discovery of the genetic basis of achondroplasia opened the door for exploration of the biochemical mechanisms that cause the underlying clinical fea-

tures of the disorder. Studies exploring these biochemical mechanisms as well as targeting the effects of the gene mutation at the cellular level are still in the developmental stage.<sup>36</sup>

This study points to the extreme importance of accurate awareness of the cardinal features of achondroplasia, proper anthropometric measurements and detailed skeletal survey as the key for accurate diagnosis, proper genetic counseling, avoidance of over diagnosis and differentiation from other types of skeletal dysplasias. Routine follow up of cases to avoid and properly manage expected complications should be considered after diagnosis. Definitive diagnosis in confusing cases and prenatal diagnosis in high risk pregnancies can be carried out by molecular analysis.

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