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1 **Fine grained facial phenotype-genotype analysis in Wolf-Hirschhorn**  
2 **syndrome**

3

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19

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22

23

1 **Summary**

2

3 Wolf–Hirschhorn syndrome is caused by anomalies of the short arm of chromosome 4.  
4 About 55% of cases are due to *de novo* terminal deletions, 40% from unbalanced  
5 translocations and 5% from other abnormalities. The facial phenotype is characterized  
6 by hypertelorism, protruding eyes, prominent glabella, broad nasal bridge and short  
7 philtrum. We used dense surface modeling and pattern recognition techniques to  
8 delineate the milder facial phenotype of individuals with a small terminal deletion  
9 (breakpoint within 4p16.3) compared to those with a large deletion (breakpoint more  
10 proximal than 4p16.3). Further fine grained facial analysis of several individuals with an  
11 atypical genotype and/or phenotype suggests that multiple genes contiguously  
12 contribute to the characteristic Wolf–Hirschhorn syndrome facial phenotype.

13

14 **Keywords**

15 Wolf-Hirschhorn syndrome; facial dysmorphism; 3D shape analysis

16

## 1 **Introduction**

2 Wolf-Hirschhorn syndrome (OMIM 194190) is a contiguous gene syndrome caused by  
3 deletion of the short arm of chromosome 4. The first report of the condition<sup>1</sup> was re-  
4 published four years later alongside another by Wolf et al<sup>2,3</sup>. Since then, more than 150  
5 patients have been reported<sup>4</sup>. Previously, approximately 75% of cases were considered  
6 to be due to *de novo* terminal deletions<sup>5,6</sup>, 13% from unbalanced translocations and the  
7 remainder from a more unusual cytogenetic abnormality. More recent studies have  
8 suggested the relative frequencies of deletions, unbalanced translocations and other  
9 causes to be 55%, 40% and 5% respectively<sup>7</sup>. Translocations between chromosomes 4  
10 and 8 occur more frequently than expected<sup>7,8,9,10,11</sup>.

11 Wolf-Hirschhorn syndrome (WHS) is characterized by pre and postnatal growth  
12 delay, microcephaly, seizures, hypotonia, developmental delay, congenital anomalies,  
13 and a recognizable facial appearance which includes: hypertelorism, protruding eyes,  
14 epicanthus, arched eyebrows, prominent nasal bridge, downturned corners of the mouth,  
15 micrognathia and short philtrum, and, with increasing age, a more prominent nose.  
16 Dental anomalies, some occurring in more than half of WHS patients, have been  
17 described: delayed eruption and hypodontia, retained primary teeth, peg-shaped teeth  
18 and taurodontism<sup>4,12</sup>. Midline defects occur in approximately 50% of cases and include  
19 cleft lip and palate and cardiac defects<sup>13</sup>.

20 There is considerable variation in the phenotypic spectrum and more recently a  
21 correlation with the size of the deletion has been recognized; children with  
22 submicroscopic deletions within 4p16.3 tend to have a milder phenotype<sup>14,15</sup>. Some  
23 patients with microdeletions within 4p16.3 were initially reported as having Pitt-  
24 Rogers-Danks syndrome, now considered to be within, and at the milder end of, the

1 WHS spectrum<sup>16,17</sup>. We use **small** and **large** respectively to refer to a terminal deletion  
2 with a break point within 4p16.3, a deletion size of about 3.1 Mb or less, and one with a  
3 break point more proximal than 4p16.3.

4 The loss of genetic material on chromosome 4 is variable with some deletions  
5 being visible using standard cytogenetic techniques while others, being submicroscopic,  
6 require the use of FISH probes for their detection. Two Wolf Hirschhorn critical  
7 regions, WHSCR1 and WHSCR2, have been identified within 4p16.3<sup>18,19</sup>. WHSCR2 is  
8 distal to, and overlaps with, WHSCR1, and three genes *LETMI*, *WHSC1* and *WHSC2*  
9 have been identified within these regions. The gene *LETMI* is thought to be associated  
10 with seizures. It has been suggested that the facial phenotype arises from loss of  
11 *WHSC1*<sup>20</sup> but a later communication<sup>21</sup> reported one patient without the facial phenotype  
12 with *WHSC1* deleted and another patient with the facial phenotype but with *WHSC1*  
13 retained. Two recent studies<sup>22,23</sup> have suggested that the fibroblast growth factor  
14 receptor *FGFRL1* is involved in the aetiology of Wolf-Hirschhorn syndrome and in  
15 particular influences craniofacial development. Figure 1A shows the relative positions  
16 of *FGFRL1*, *LETMI*, *WHSC1* and *WHSC2* in 4p16.3, corresponding approximately to  
17 the terminal 3.1Mb region. In WHS patients with oligodontia, the gene *MSXI* has been  
18 found to be deleted<sup>24</sup>. *MSXI* lies proximally to 4p16.3.

19 Wiczorek and collaborators previously reported a correlation in WHS between  
20 postnatal head circumference and deletion size<sup>14</sup>. The original aim of this study was to  
21 analyse the effects of deletion size on facial morphology in WHS. In order to test this  
22 hypothesis in an objective manner, we undertook a quantitative facial analysis using 3D  
23 photographs and dense surface modelling techniques<sup>25,26</sup>. Previously, studies using 3D  
24 dense surface models (DSMs) of face shape have delineated common facial features in a

1 range of neurodevelopmental conditions, often, in addition, establishing accurate  
2 discriminating characteristics or assisting the determination of phenotype-genotype  
3 correlations<sup>26,27,28,29,30,31,32,33,34</sup>. We have shown that DSM based analysis provides a  
4 very accurate instrument for classifying faces or facial regions along the control-WHS  
5 spectrum. In addition, for the first time, we have used DSMs to construct mean face  
6 surfaces matched for age and size for fine grained analysis of six individuals so that  
7 subtle face shape differences can be related to the underlying genotype. Our findings  
8 support the hypothesis that multiple genes contribute to the facial phenotype of Wolf-  
9 Hirschhorn syndrome in a contiguous fashion.

10

### 11 **Subjects and Materials**

12 3D face images of individuals with WHS were collected using a commercial  
13 photogrammetric device at family support group meetings in the UK and USA, and  
14 during a scanning trip to Italy. The accuracy of such 3D imaging devices has been  
15 shown to be highly reliable<sup>35</sup>. Some images were unusable because the coverage of the  
16 face was incomplete or the subject was not sufficiently co-operative to capture a usable  
17 image. In addition, several hundred images of unrelated, unaffected individuals of a  
18 wide age range (3 months to over 70 years) were drawn from an existing collection with  
19 a view to making comparisons between the WHS group and matched controls. All  
20 images were collected in association with informed written consent and research ethics  
21 approval (UCLH:JREC00/E042; Sheffield: MREC/03/4/022). Details of the specific 4p  
22 abnormality were obtained from clinical records.

23 In the analysis of younger subjects, the WHS dataset comprised 100  
24 individuals of white, European descent (1.0 - 19.5 yrs; mean 7.9 yrs) with 81 confirmed

1 deletions of 4p with known breakpoints (2 interstitial; 23 small (3.1Mb or less); 28 large  
2 (more than 3.1 Mb); 28 translocations) and 19 with breakpoints reported as 4p16 which  
3 could not be further subclassified. These were matched ethnically with a control group  
4 of 200 individuals between (0.2 - 20.7 yrs; mean 8.6 yrs). A small group of 5 children  
5 under 5 yrs with an ethnic background other than white European was also included in  
6 the study. For the analysis of older subjects, we used an overlapping set of 43  
7 individuals with WHS between 9 and 32.3 yrs (mean 15.6 yrs) and 141 controls  
8 between 9 and 29.5 yrs (mean 16.1 yrs). Finally, six individual cases were subjected to  
9 more refined facial analysis: one with a small deletion and a typical WHS phenotype;  
10 his mother with an even smaller deletion; and, four others without the classical WHS  
11 facial features. Approximately 400 control faces were used for the fine grained analysis.

#### 12 Cases T1 and MT1

13 T1 and MT1 were first reported by Faravelli et al<sup>36</sup> where full details can be found. At  
14 the time of this study they were 18.1 yrs and 57.5 yrs. T1 had a 2.8 Mb 4p terminal  
15 deletion including both critical regions and a typical phenotype for his diagnosis of  
16 WHS. In contrast, his mother, MT1, had a 1.35 Mb to 1.47 Mb terminal deletion distal  
17 to both critical regions and removing only *FGFRL1*. MT1's face was considered WHS-  
18 like with hypertelorism, high nasal bridge, large protruding eyes and down slanting  
19 palpebral fissures. Her cognitive abilities were within lower limits of normal. Her motor  
20 development was normal and she experienced a few febrile seizures before the age of  
21 five. When analyzing younger subjects, we included T1 in training sets as someone  
22 with a small deletion and typical phenotype. When analyzing older subjects, we  
23 excluded T1 and MT1 from model generation so we could compare their faces blinded  
24 and detect differences due to distinct genotypes.

1

2 Case A1

3 Case A1 was a 1.8 yr old male patient with an interstitial deletion of approximately 4.3  
4 Mb removing *LETMI*, *WHSC1* and *WHSC2* but retaining *FGFRL1*. Intrauterine growth  
5 retardation was noted during pregnancy. Delivery was at 39 weeks and birth weight was  
6 2.5kg. Parents were concerned about his weight gain at age 4 months and at 6 months an  
7 atrial septal defect not requiring treatment was detected. Feeding continued to be  
8 difficult and a gastrostomy tube was used from 10 months to 3 years. He sat at 1 year  
9 and at 3.8 years was walking if held by both hands. Following removal of tonsils and  
10 adenoids at 1.9 years, he spoke single words and now uses short phrases. He has duplex  
11 ureters, no reflux and no seizures to date.

12

13 Case A2

14 Case A2 was a 4.6 year old female patient published as one of two cases of 4p16.3  
15 deletions excluding both WHS critical regions. *FGFRL1* and a portion of the *LETMI*  
16 gene were absent in a terminal deletion of approximately 1.78 Mb distal to WHSCR1  
17 with a breakpoint near or within the distal boundary of WHSCR2. She had growth  
18 retardation, development delay, lacked typical WHS facial features but was described as  
19 having a prominent forehead, mild telecanthus and normal philtral length<sup>37</sup>. At 6 months  
20 of age myoclonic jerking was investigated and clinical episodes of atypical absence  
21 seizures have recurred.

22

23 Cases A3 and MA3

1 Case A3 was a 6.3 year old female patient with a maternally inherited terminal deletion  
2 of 160Kb to 400Kb. Following concern over fetal growth, she was delivered by  
3 Caesarean section at 38 weeks. Her head circumference was around the 0.4<sup>th</sup> centile but  
4 then fell away from the centile whereas her weight was between the 2<sup>nd</sup> and 9<sup>th</sup> centiles.  
5 She did not have a history of seizures but required surgical repair of an atrial septal  
6 defect and a right inguinal hernia. Her development was delayed; she attended  
7 mainstream education with 1:1 support. Her parents were healthy and had normal head  
8 circumferences. The deleted region had not been reported within the Database of  
9 Genomic Variation and was not observed in more than 400 control cases. Therefore, her  
10 deletion may be a rare benign copy number variation, a conclusion consistent with the  
11 absence of a clinical phenotype in the mother (MA3). However, a contribution to A3's  
12 phenotype cannot be excluded.

13 Figure 1A illustrates the 3.1Mb terminal region of chromosome 4p  
14 corresponding approximately to 4p16.3 with annotations showing the WHS critical  
15 regions, important genes, and the deletions of the selected cases T1, MT1, A1-A3, and  
16 MA3. Also represented in the figure is the case where a role for *FGFRL1* in the WHS  
17 facial phenotype was first proposed<sup>22</sup>. This patient has a terminal deletion with a  
18 breakpoint in between those of MT1 and A2. Supplementary table ST1 gives more  
19 detailed descriptions of the breakpoints for these six individuals.

20

## 21 **Methods**

### 22 *Comparison of linear regressions*

23 Linear regressions were undertaken for various facial measurements and DSM based  
24 markers against age. The approach of Wuensch et al<sup>40</sup> was used to determine significant

1 differences in slope and/or intercept in comparisons of separate regressions for control  
2 and WHS subgroups.

3

#### 4 *Image preparation*

5 Each captured face surface was annotated manually by one individual (PH) at 22  
6 anatomical locations: left and right endocanthion, exocanthion, palpebrale superius,  
7 palpebrale inferius, otobasion inferius, crista philtrum, cheilion, and alare; nasion,  
8 pronasale, subnasale, labiale superius, labiale inferius and gnathion (Supplementary  
9 Figure S1). These landmarks have previously been studied for their reproducibility and  
10 accuracy<sup>38</sup>. Generally speaking, landmarks on the lips and eyes are the most  
11 reproducible. All landmarking and dense surface model analyses were undertaken using  
12 software developed in-house<sup>30,39</sup>.

13

#### 14 *DSM building and closest mean classification*

15 DSMs were constructed using techniques described elsewhere<sup>25,26</sup>. A DSM refers to the  
16 set of principal components (PCs) or PCA modes accounting for the shape variation in  
17 the surfaces included. For a set of faces with wide age variation, the first mode of  
18 variation (PC1) follows the shape of a typical growth curve and is highly correlated with  
19 age. With closest mean classification, average faces are computed for the control and  
20 affected subgroups and each face is classified according to which average is closest  
21 using the DSM representation. For “blinded” or unseen testing, we randomly generated  
22 twenty 90%-10% training-test set pairs sampled in a stratified fashion from the control  
23 and WHS groups. We used the training sets to build DSMs and the test sets for unseen  
24 or blinded classification. The accuracy of classification to control or WHS group was

1 estimated as the mean of the areas under the (20) receiver operating characteristics  
2 (ROC) curves arising from the unseen testing. Alongside closest mean classification, a  
3 useful measure defining outlier status is the distance of a face from the line joining  
4 unaffected and affected means in the DSM representation.

5         PCA models are used generally to reduce dimensionality of complex multi-  
6 variate datasets. In such models, the modes are listed in terms of largest to smallest  
7 variation coverage. In pattern recognition, a typical approach is to retain modes  
8 covering 95%-99% of shape variation on the assumption that omitted modes represent  
9 noise. To optimise discrimination, we performed classification testing for all possible  
10 leading subsequences of modes in the models and for each of three pattern recognition  
11 classification algorithms - closest mean (CM); linear discriminant analysis (LDA); and,  
12 support vector machines (SVM).

13

## 14 **Results**

15 *Facial growth in WHS is significantly delayed but does not correlate with deletion size*  
16 PC1 in a DSM for a reduced face patch (no ears) computed for the control and WHS  
17 subjects under 20 yrs is highly correlated with age (Figure S3). Separate linear  
18 regressions of PC1 against age for control and WHS subgroups highlight both delay and  
19 diminished rate of facial growth in WHS. The difference in slope was statistically  
20 significant ( $t=2.5724$ ,  $df=296$ ,  $p=0.0107$ ) and the difference in intercept highly  
21 significant ( $t=8.6024$ ,  $df=296$ ,  $p<0.0001$ ). These results are predictably consistent with  
22 the universal clinical observation of reduced birth size and increasing size difference  
23 throughout the life of a child with WHS. Restricting the facial growth analysis to  
24 individuals with small and large deletions or translocations does not suggest any

1 correlation between facial growth and deletion size (Figure 3). The inclusion of A1-A3  
2 in the figure demonstrated that each had delay in facial growth.

3

4 *Facial dysmorphology in WHS is visualized effectively using static and dynamic*  
5 *comparisons*

6 Comparisons of the mean faces of the WHS and control subgroups confirmed many of  
7 the well documented facial characteristics. In Figure 2A, the two mean faces are shown  
8 to the same scale using surface shape alone. A combined shape and facial appearance  
9 DSM provided similar but more photorealistic mean surfaces (Figure 2B). The third  
10 comparison is a heat map of the mean WHS face reflecting location differences between  
11 corresponding points on the mean control and WHS faces along a normal to the mean  
12 WHS face surface. The red–green–blue spectrum depicts inward–null–outward  
13 displacement along the surface normal (Figure 2C). The abundance of red clearly  
14 demonstrates how the comparison is overwhelmed by the gross size difference in the  
15 two means, despite their corresponding mean ages differing by only months. By  
16 adjusting for size using the ratio of nasion-gnathion length for each mean surface, a  
17 shape only comparison was possible and by focusing on position differences parallel to  
18 three orthogonal axes, more subtle shape differences were revealed. For example, a  
19 comparison of left-right horizontal differences in corresponding points in Figure 2D  
20 indicates relative hypertelorism and the stronger colouring at the outer canthi indicates  
21 relatively wider palpebral fissures in WHS. Relative to face length (used in the  
22 resizing), the WHS mean face is also shown to have a wider zygomatic arch, to be  
23 broader at the tragion level but to have a narrower gonial width reflecting a much  
24 smaller mandible. The vertical axial comparison of Figure 2E emphasises the upward

1 sweep of the supra-orbit and associated eyebrow arching; the shortened philtrum (blue  
2 generally on upper lip) and concave curling of the upper lip (dark blue at centre of upper  
3 lip); reduced anterior mandible height (blue on chin); and, low set ear position (red on  
4 ears). The prominent glabella, proptosis and reduced mandible size are all confirmed in  
5 the depth-wise comparison of Figure 2F.

6           Similar visualisations are achievable by building DSMs for patches of  
7 face surface. More subtle shape differences, swamped by larger ones in a full face  
8 model, are revealed by focusing on smaller regions of the face. For example, from a  
9 DSM for a nasal patch, the size (nasion-subnasale) adjusted shape comparison  
10 emphasises a broader nasal root and bridge (Figure 2G); longer nose and vertically  
11 diminished alae (Figure 2H) ; and, depth-wise flattening of the nose tip (Figure 2I). A  
12 comparison with the cartoon in (Figure 2J-2K) further supports the hypothesis that these  
13 nose shape differences are very much due to altered positioning and shape of the  
14 underlying nasal cartilages (especially Figure 2G).

15           Finally, dynamic morphs between the control and WHS mean faces  
16 very clearly demonstrate the important differences in face shape either with size  
17 retained or more emphatically when it is adjusted for (see supplementary material at  
18 journal web site).

19

#### 20 *Facial dysmorphism in WHS is milder for individuals with small deletions*

21 In a single DSM for a reduced face patch (without ears) for 200 controls and 100  
22 individuals with WHS and under 20 years, we calculated the normalised position of  
23 each constituent face between the mean control and WHS faces as described earlier.  
24 Figure 3B shows the classification position of the faces of individuals with small and

1 large deletions relative to the overall control and WHS average faces normalized to the  
2 ends of the  $x$ -axis interval  $[-1, +1]$ . The  $y$ -axis represents generalized Euclidean distance  
3 between a face and the hyperline joining the two means in the DSM, also defined  
4 earlier. Thus, position on the  $x$ -axis reflects similarity to one of the means, whereas  
5 position on the  $y$ -axis reflects difference from both means with higher values reflecting  
6 outlier status for one or both subgroups. The shaded convex hulls show that 50% of the  
7 small deletion subgroup are closer to the control mean than the large deletion subgroup;  
8 that 50% or so of the large deletion subgroup overlap the small deletion subgroup; and,  
9 that the remainder are either more extreme than the mean WHS (further out on the  $x$ -  
10 axis) or more outlying from both means (further out on the  $y$ -axis).

11

12 *DSM analysis supports high levels of discrimination between controls and individuals*  
13 *with WHS*

14 We used 20 training-unseen test set pairs for the multi-folded face classification of the  
15 younger study group of Caucasian individuals. The graphs in supplementary figure S2  
16 show how the estimated discrimination accuracy varies according to the number of  
17 modes retained in the DSMs and to the pattern recognition algorithm employed - closest  
18 mean (CM); linear discriminant analysis (LDA); and, support vector machines (SVM).  
19 Supplementary table 1 summarises the best discrimination rates for the face (without  
20 ears) and patches around the eyes, nose and mouth and for each of the three pattern  
21 recognition algorithms. Almost perfect classification was obtained in each case. An  
22 analogous multi-folded classification analysis of the older subjects produced similar  
23 almost perfect rates of discrimination for each algorithm.

1                   We also classified the face shape of 5 affected children under 5.2 yrs  
2 with an ethnic origin other than white European, four had large deletions and one a  
3 small deletion. There were insufficient numbers of ethnically matched subjects for a  
4 separate classificatory cross-validation so instead we used the models based on  
5 European subjects. The difference in ethnic background had no effect on the success of  
6 the face shape classification.

7

8 *Fine grained facial phenotype-genotype analysis of six selected individuals*

9 To prepare for visual analysis of the faces of the selected cases (T1, MT1, A1-A3,  
10 MA3) we computed running means of contiguously aged same sex subgroups of size 40  
11 for 400 or so controls. We then selected same sex running mean subgroups whose mean  
12 ages matched those of the selected cases to compute age/sex matched mean faces. Using  
13 nasion-gnathion length, we then made size-adjusted comparisons analogous to the size-  
14 adjusted “control mean-WHS mean” comparisons described above. The heat map  
15 comparisons of these patients to age-sex-size matched means in Figure 4 need careful  
16 comparison with Figures 2D-2F.

17                   FIGs 4D-4F for subject A2 show similarities to FIGs 2D-2F: some  
18 hypertelorism (red/blue patches on eyes in 4D); wider palpebral fissures (stronger  
19 colouring at exocanthi in 4D); some shortening of the philtrum (blue on upper lip in  
20 4E); a prominent glabella (blue on forehead in 4F); and, a little (asymmetric) proptosis  
21 (blue on eyes in 4F). For A1, there is no hypertelorism (Figure 4A), a slight vertical  
22 displacement in the supra-orbital region (4B), no reduction in philtrum length (4B), no  
23 proptosis (4C) and a little asymmetric prominence of the glabella (4C). A3 shows no  
24 signs of the WHS facial phenotype (4G-4I) - the shortening of the philtrum (4H) was

1 likely due to facial expression stretching the upper lip. The mother of A3 , MA3, shows  
2 no WHS-like features (4P-4R). As expected from his deletion and WHS diagnosis, T1  
3 shows hypertelorism (4J); a prominent nasal bridge (4L); shortened/curled philtrum  
4 (4K); an upsweep of the supra-orbital ridge (4K); and, proptosis and prominence of the  
5 nasal bridge (4L). In contrast, his mother, MT1, showed no hypertelorism (4M); an  
6 upward sweep of the supra-orbital ridge (4N); and, a prominent nasal bridge (4O). The  
7 proptosis and prominence of the glabella that are detectable by eye were disguised by  
8 mild mid-facial hypoplasia and fullness of the cheeks of the face (4O). This resulted in  
9 the flattened areas being painted red and the periorbit green – but still reflected relative  
10 proptosis. Specific size adjusted patch comparisons of the periorbital and perinasal  
11 regions also highlight the proptosis and the glabella prominence (not shown).

12 To complement the visual analysis, the DSMs constructed for the two  
13 cross validations conducted for younger and older subjects were re-used to classify the  
14 face/face patches of the six selected cases in a blinded or unseen fashion. Their average  
15 closest mean classification positions for face, periorbital and perinasal patches with 95%  
16 confidence intervals are shown in Figure 1 along with a summary of the genes affected  
17 by their respective deletions. MA3 is classified close to the mean control face and even  
18 further into the control groups for the periorbital and perinasal patches. A3 is classified  
19 close to the control-WHS borderline suggesting some facial dysmorphism overall but  
20 with much more control-like than WHS-like features for the smaller face patches. MT1,  
21 whose deletion removes only *FGFRL1*, is also classified facially close to the control-  
22 WHS border. But consistent with the size adjusted surface comparisons above, her  
23 periorbital patch is classified as more WHS-like. The interstitial deletion of A1,  
24 affecting *LETMI* and *WHSCI*, results in classification positions on or just control side

1 of the control-WHS borderline. This is consistent with the size adjusted surface  
2 comparisons suggesting very mild dysmorphism with more control-like than WHS-like  
3 features. A2's loss/disruption of *FGFRL1* and *LETMI* results in classification positions  
4 consistent with the mild hypertelorism and glabella prominence identified in the size  
5 adjusted surface patch comparisons. Finally, the loss of *FGRL1*, *LETMI* and *WHSC1* in  
6 T1's deletion results in classifications on or close to the mean WHS position.

7

## 8 **Discussion**

9 Mouse models of the two WHS critical regions have failed to recapitulate the classical  
10 phenotype of WHS. The human gene *FGFRL1*, more distal than both critical regions,  
11 was implicated recently in the craniofacial phenotype of a single patient<sup>22</sup> whose  
12 terminal deletion has a breakpoint between those of A2 and MT1. A targeted deletion of  
13 the mouse gene *Fgfr1l* recapitulated much of the WHS phenotype<sup>23</sup>. *Fgfr1l* is expressed  
14 in cartilaginous structures such as primordia of bones and permanent cartilage of the  
15 trachea, ribs and nose<sup>41</sup>. Our DSM analysis of a nasal patch of the face surface (Figure  
16 2) detected altered shape that appears to coincide with nasal cartilage structures,  
17 especially those of the lower nasal cartilage. These findings support the hypothesis that  
18 *FGFRL1* is influential in the WHS facial phenotype.

19 Subjects MA3 and A3 retain all the highlighted genes and have no significant  
20 features that would be given a diagnosis of WHS. The interstitial deletion of patient A1  
21 removes *LETMI*, *WHSC1* and *WHSC2*, but retains *FGFRL1*. Our analysis suggests that  
22 this patient does not have WHS facial characteristics but does have some mildly  
23 dysmorphic features. The terminal deletion of patient A2 spares *WHSC1* and *WHSC2*,  
24 interferes with the distal end of *LETMI* and removes *FGFRL1*. The fine grained facial

1 analysis suggests that patient A2 has some isolated facial features that are also part of  
2 the WHS facial phenotype. A1 and A2 are in a grey area but could not be given a  
3 diagnosis of WHS. MT1's deletion involves *FGFRL1* alone but does appear to produce  
4 some WHS facial characteristics, as was suggested for the patient in (Engbers et al,  
5 2009) whom we have not analysed. Thus, the loss or disruption of *FGFRL1* or *WHSCI*  
6 alone does affect facial morphology, with *FGFRL1* possibly having a slightly greater  
7 effect. But when the deletion is a contiguous sequence including both *FGFRL1* and  
8 *WHSCI*, as with patient T1, the full complement of the WHS facial characteristics  
9 results. T1 would certainly be given a diagnosis of WHS but MT1 would not.

10 In this study, we first demonstrated that small terminal deletions with a  
11 breakpoint within 4p16.3 are associated with milder facial dysmorphology than large  
12 deletions. This was achieved using dense surface models to develop sensitive and  
13 accurate visualisation and quantitative tools for discerning control-WHS face shape  
14 difference. Armed with these instruments we undertook fine grained facial analysis of  
15 six selected patients suggesting that WHS facial features are due to the combined  
16 deletion of multiple genes adding further weight to the hypothesis that WHS is a true  
17 contiguous gene disorder as previously suggested<sup>20</sup>.

18 Although facial dysmorphism in WHS can be clinically recognizable, an  
19 objective, quantitative evaluation is particularly valuable when assessing phenotypically  
20 or genotypically unusual cases. Such individuals are likely to be particularly informative  
21 in genotype-phenotype correlation analysis and the clarification of the influence of  
22 individual genes. In the future, DSM based analysis will enable the recognition of WHS  
23 facial characteristics in patients without 4p deletions. Genetic/genomic analysis of such

1 patients may identify mutations and in combination with detailed facial analysis enable  
2 unequivocal identification of causative genes.

3

#### 4 **Supplemental Data**

5 The supplemental data comprises a brief summary of dental findings, two tables, two  
6 video animations and 3 figures.

7

#### 8 **Acknowledgements**

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10 study and allowed their faces to be scanned. The WHS family support groups of the UK  
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13 financial support that enabled several generations of 3D cameras to be purchased and  
14 many field trips to be undertaken. We also thank Dr Helen Cox for comments on the  
15 manuscript and the Sheffield Children's appeal for travel grants to OQ.

16

#### 17 **Conflict of interest**

18

19 The authors declare no conflict of interest.

20

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10

1 **Figure Titles and Legends**

2 **Figure 1**

3 **A:** The terminal 3.1Mb region of chromosome 4p corresponding approximately to  
4 4p16.3 is shown annotated by the two critical regions WHSCR1 and WHSCR2,  
5 and four genes (*FGFRL1*, *LETM1*, *WHSC1* and *WHSC2*). The five black bands  
6 depict the deletions of patients T1, MT1, A1-A3, MA3 selected for fine grained  
7 facial analysis and the grey band that of a previously cited patient linked with  
8 *FGFRL1*.

9 **B:** Mean face patch classification positions for the six selected patients with  
10 reference to the particular genes affected by their deletion. (X means gene is  
11 deleted or curtailed)

12

13 **Figure 2**

14 **A:** comparisons of the mean faces of the control and WHS subgroups computed from  
15 a shape-only DSM;

16 **B:** comparisons of the mean faces of the control and WHS subgroups computed from  
17 a combined shape and appearance DSM;

18 **C:** a heat map comparison of the raw mean faces reflecting displacement normal to  
19 the surface of the WHS mean;

20 **D-F:** size adjusted heat map comparisons parallel to three orthogonal axes;

21 **G-I:** heat map comparisons of mean WHS nose surface to mean control nose surface  
22 with size adjustment and reflecting displacement along three orthogonal axes;

23 **J-K:** cartoons showing nasal cartilage substructures.

24

1 **Figure 3**

2 **A:** A scatter of age against PC1 is shown for a single DSM for a reduced face patch  
3 (no ears) for both the control and WHS subgroups. The scatter is restricted to  
4 those individuals known to have small or large deletions or unbalanced  
5 translocations. The younger selected patients A1-A3 are also represented.

6 **B:** Closest mean classification positions of all controls (n=200) and those individuals  
7 with WHS who have a small or large deletion (n=51).  
8

9 **Figure 4:**

10 **A-I:** Heat maps comparing the younger subjects with an age matched average face  
11 from the running mean sequence of 40 age-contiguous control faces. Three axial  
12 comparisons are given.  
13

14 **J-R:** Heat maps comparing the older selected subjects with an age matched average  
15 face from the running mean sequence of 40 age-contiguous control faces. Three  
16 axial comparisons are given.  
17

18 **SUPPLEMENTARY FIGURES AND TABLES**

19 **Figure S1**

20 Positions of the 22 landmarks used to annotate face surfaces.  
21

22 **Figure S2**

23 Each graph shows the classification accuracy resulting from a 20-fold cross validation  
24 as the mean area under the 20 associated ROC curves against the number of modes

1 retained in the associated DSM. (DSM= dense surface model; ROC= receiver operating  
2 characteristic; PCA = principal component analysis' CM = closest mean; LDA = linear  
3 discriminant analysis; SVM = support vector machines)

4

5 **Figure S3**

6 A scatter of age against PC1 is shown for a single DSM for a reduced face patch (no  
7 ears) for both the control and WHS subgroups. The scatter is annotated by separate  
8 linear regression lines for each subgroup.

9

1 **Table ST1:**

2 **Legend:** Best classification rates for control-WHS facial discrimination using three  
 3 different pattern recognition techniques (CM = closest mean; LDA = linear discriminant  
 4 analysis; SVM = support vector machines) for both younger subjects.

5

	<b>FACE</b>	<b>EYES</b>	<b>NOSE</b>	<b>MOUTH</b>
CM	0.999	0.992	0.998	0.991
LDA	1.000	0.994	0.998	0.989
SVM	1.000	0.997	0.998	0.992

6

7

8

9 **Table ST2:**

10

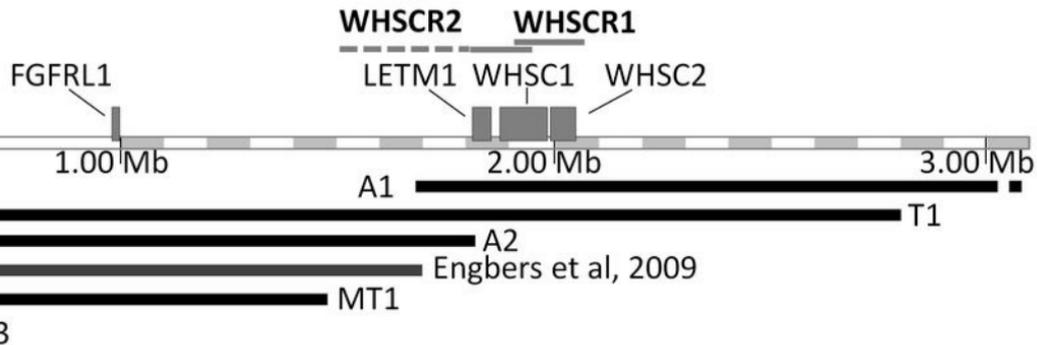
11 **Legend:** Detailed description of deletions for atypical cases

12

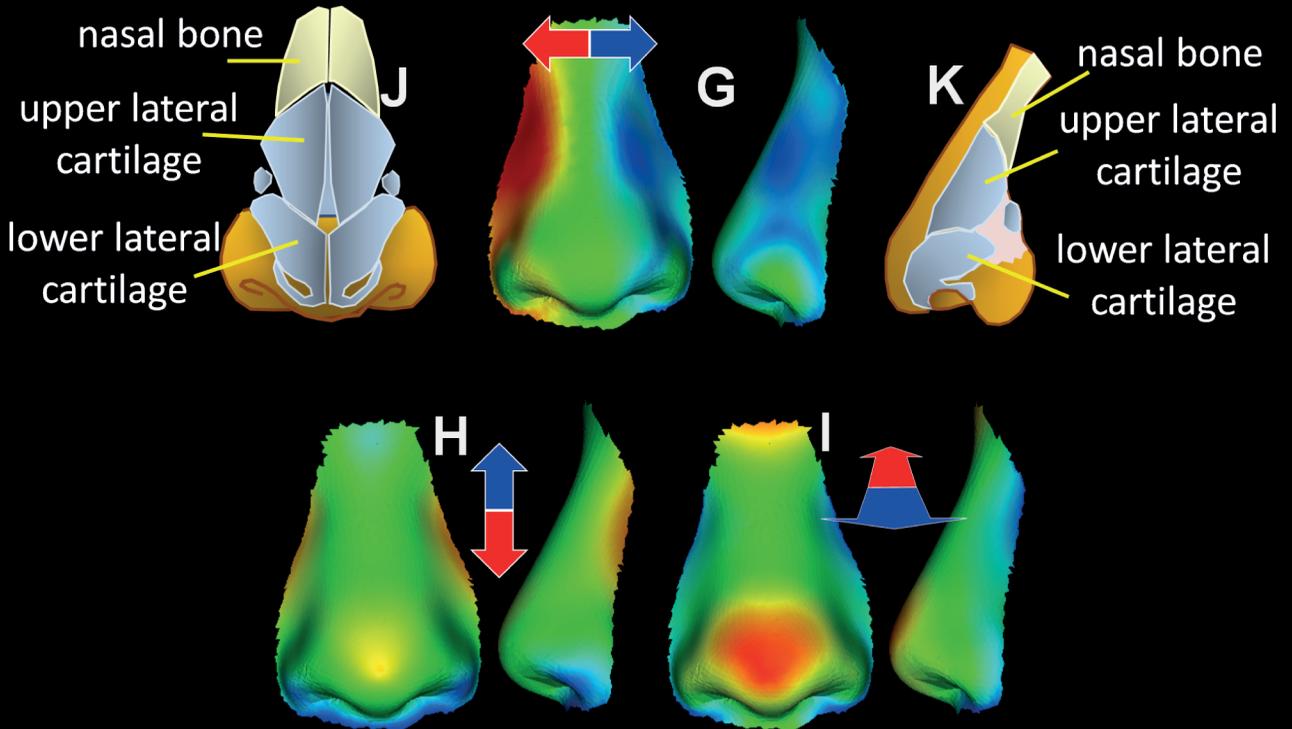
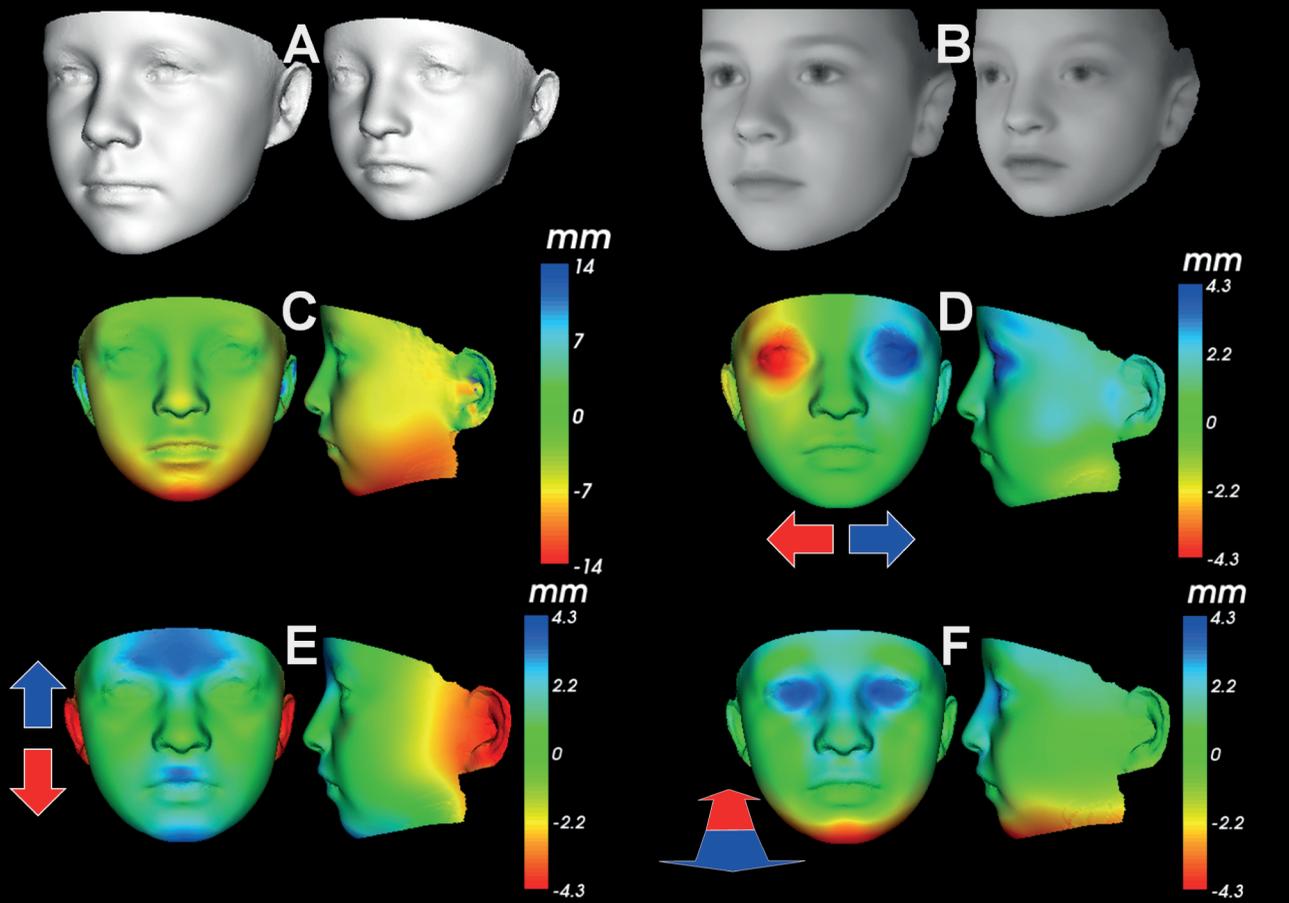
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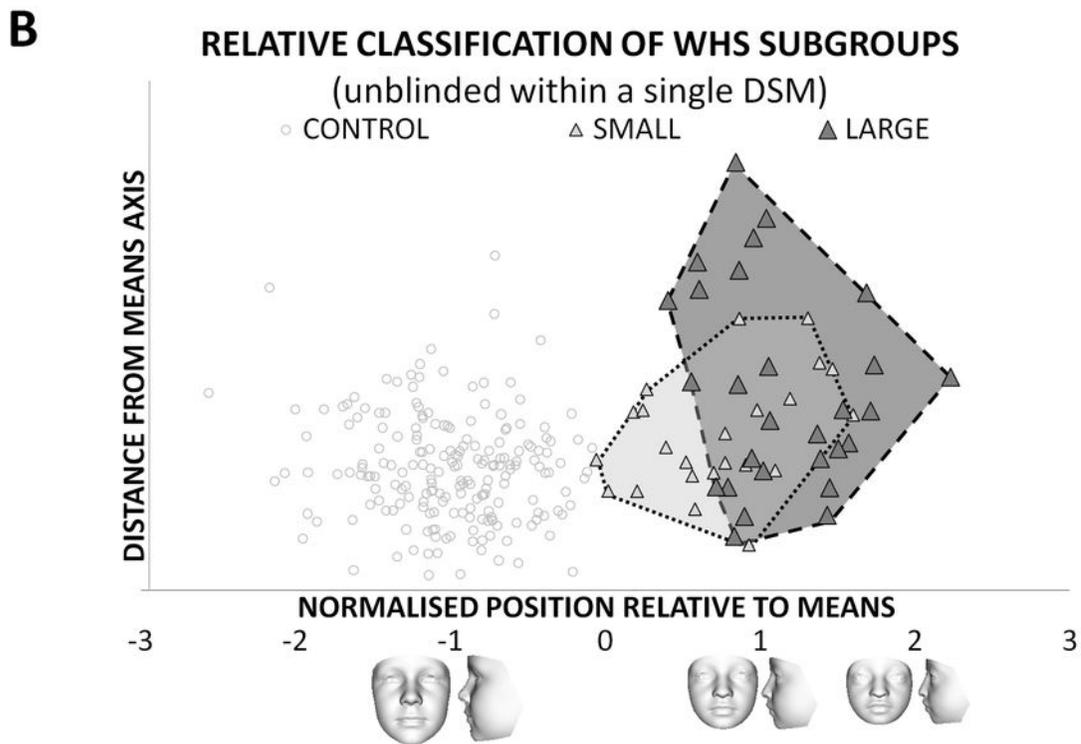
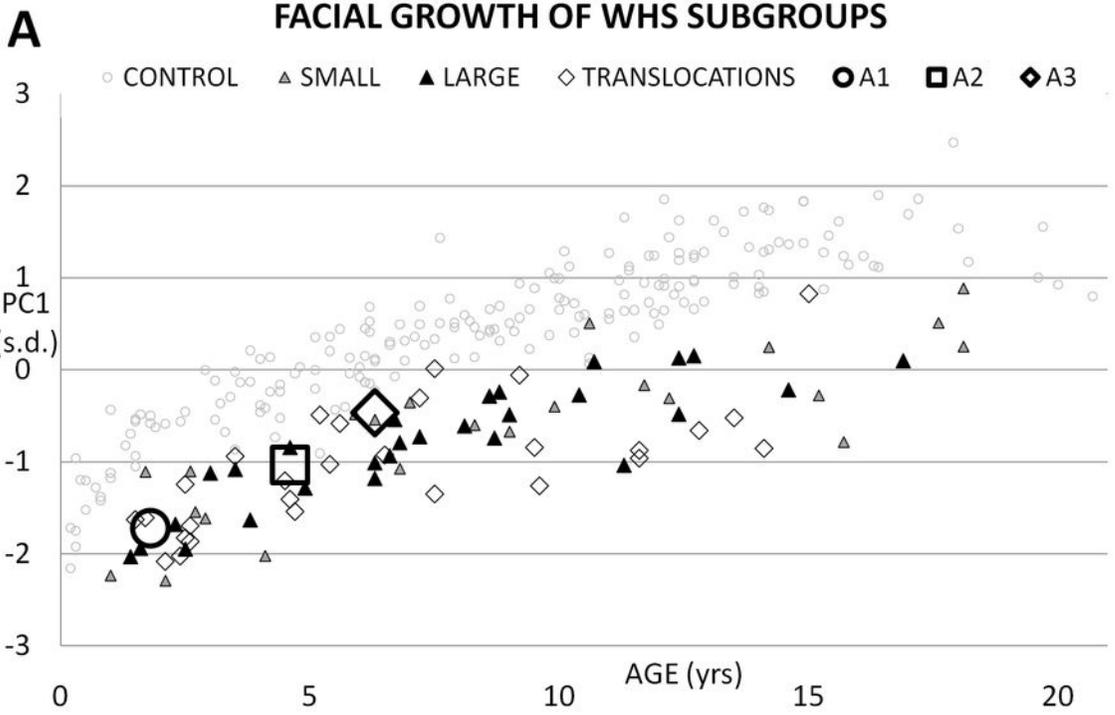
<b>Case</b>	<b>Deletion</b>
A1	arr cgh 4p16.2-16.3 (RP11-241P10->RP11933C4) x1
A2	arr cgh 4p16.3 (pter-> 1,780,000) x1
A3	arr cgh 4p16.3 (RP11-46D1) x1 mat
T1	46,XY .ish del(4p16.3) (F26-; IS28-; D4S96-; D4S3327-; D4S43-; D4S182-; D4S180x2)
MT1	46,XX .ish del(4p16.3) (F26-;IS28-;D4S96-;FGFR3x2;D4S3327x2; D4S43x2;D4S182x2;D4S180x2)
A3	arr cgh 4p16.3 (RP11-46D1) x1

14

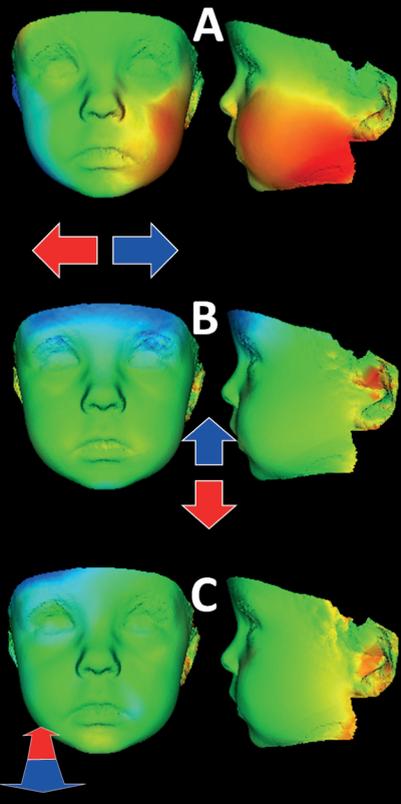
**A****B**

	<b>F G F R L 1</b>	<b>L E T M 1</b>	<b>W H S C 1</b>	 mean control	 mean whs	 mean control	 mean whs	 mean control	 mean whs
<b>T1</b>	X	X	X		⊕		⊖		⊕
<b>A2</b>	X	X			⊕		⊖		⊕
<b>A1</b>		X	X		⊕		⊖		⊖
<b>MT1</b>	X				⊖		⊖		⊕
<b>MA3, A3</b>				⊕	⊖	⊖	⊖	⊖	⊖

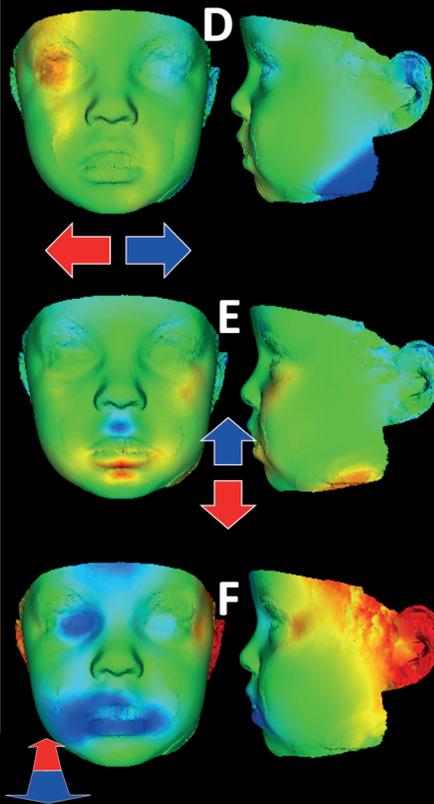




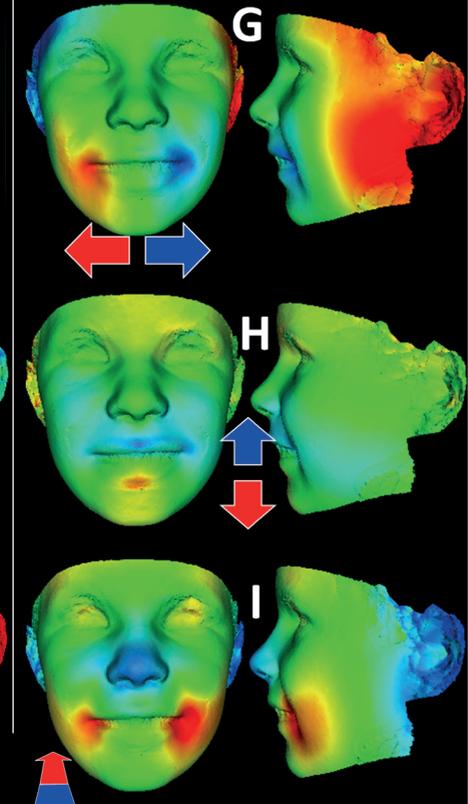
Atypical patient A1



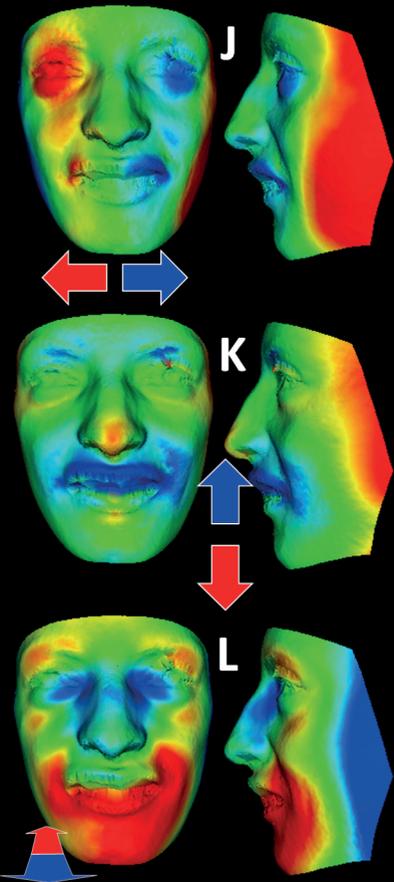
Atypical patient A2



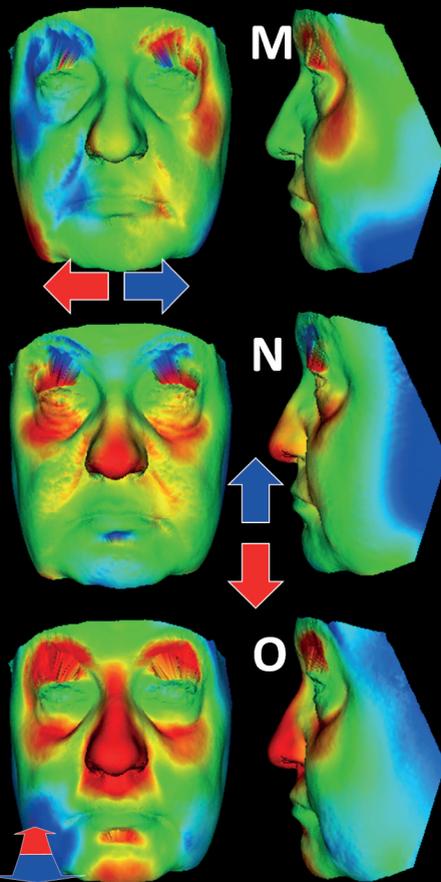
Atypical patient A3



T1 (typical phenotype)



MT1 (mother of T1)



MA3 (mother of A3)

