The American Journal of Human Genetics, Volume *104*

Supplemental Data

Complex Compound Inheritance of Lethal Lung Developmental Disorders Due to Disruption of the TBX-FGF Pathway

Justyna A. Karolak, Marie Vincent, Gail Deutsch, Tomasz Gambin, Benjamin Cogné, Olivier Pichon, Francesco Vetrini, Heather C. Mefford, Jennifer N. Dines, Katie Golden-Grant, Katrina Dipple, Amanda S. Freed, Kathleen A. Leppig, Megan Dishop, David Mowat, Bruce Bennetts, Andrew J. Gifford, Martin A. Weber, Anna F. Lee, Cornelius F. Boerkoel, Tina M. Bartell, Catherine Ward-Melver, Thomas Besnard, Florence Petit, Iben Bache, Zeynep Tümer, Marie Denis-Musquer, Madeleine Joubert, Jelena Martinovic, Claire Bénéteau, Arnaud Molin, Dominique Carles, Gwenaelle André, Eric Bieth, Nicolas Chassaing, Louise Devisme, Lara Chalabreysse, Laurent Pasquier, Véronique Secq, Massimiliano Don, Maria Orsaria, Chantal Missirian, Jérémie Mortreux, Damien Sanlaville, Linda Pons, Sébastien Küry, Stéphane Bézieau, Jean-Michel Liet, Nicolas Joram, Tiphaine Bihouée, Daryl A. Scott, Chester W. Brown, Fernando Scaglia, Anne Chun-Hui Tsai, Dorothy K. Grange, John A. Phillips 3rd, Jean P. Pfotenhauer, Shalini N. Jhangiani, Claudia G. Gonzaga-Jauregui, Wendy K. Chung, Galen M. Schauer, Mark H. Lipson, Catherine L. Mercer, Arie van Haeringen, Qian Liu, Edwina Popek, Zeynep H. Coban Akdemir, James R. Lupski, Przemyslaw Szafranski, Bertrand Isidor, Cedric Le Caignec, and Paweł Stankiewicz

Case Reports

Subject P003 was a French girl, born to non-consanguineous parents, after an uneventful pregnancy. She had three healthy siblings and there was no familial history. Delivery occurred at 40 weeks gestational age with normal birth parameters (3110 g, 48 cm, 33.5 cm). She immediately presented with severe respiratory distress and pulmonary arterial hypertension and died at 1 day of life despite active neonatal resuscitation. Autopsy showed major lung hypoplasia [lung weight/body-weight ratio (LW/BW) was 0.008)], an early arrest of pulmonary development at pseudoglandular stage with absence of alveoli and saccule, and disorganization of pulmonary architecture. These areas were adjacent to subpleural areas with near normal alveoli. Further examination showed areas of epidermal atrophy, secondary to neonatal care. Histological review concluded marked variation with acinar dysplasia (AcDys) ranging to near normal.

Subject P006 was a girl born at 38 week. The autopsy revealed the low lung weight (26.8 g), while the expected weight for that age is 40.6 g. The right lung had three lobes and the left two. Both lungs appeared small and they had a red color and "meaty" consistency. No crepitation was felt on palpation. The larynx, trachea, and bronchi were patent. In the microscopic analysis sections showed budding, branching, and interconnected large irregular bronchial structures with variable amounts of cartilage as well as alveolar ducts. These spaces contained amniotic squamous cells. The bronchi and alveolar ducts were set in a large amount of mesenchymal tissue and were separated into smaller lobules by bands of fibrocollagenous tissue. No true alveoli were seen. Blood vessels were thickened and some show fibrinoid necrosis and perivascular hemorrhage.

Subject P009 was a female fetus, from French unrelated parents, with a healthy brother. The pregnancy has been terminated at 32 weeks because of severe lung hypoplasia and pulmonary arteria hypoplasia diagnosed during ultrasound follow-up. Fetopathological examination confirmed lung hypoplasia (LW/BW=0.008), associated with fibrosis and an arrest of maturation at pseudoglandular to canalicular stage. Histological review concluded AcDys.

Subject P012 was a French girl, born to non-consanguineous parents with a healthy sister and no familial history. Moderate intrauterine growth retardation (IUGR) was noticed during pregnancy, and delivery occurred at 40 weeks with a birth weight at 2580 g (<3 centile). The infant died at 30 minutes of life due to respiratory failure and pulmonary hypertension despite intensive reanimation (intubation ventilation). Autopsy was performed and showed lung hypoplasia (LW/BW=0.007), associated with few bronchioalveolar endings and rare alveoli. In addition, she had renal hypoplasia and atrial septal defect. Histological review concluded AcDys.

Subject P015 (sibling of **subject P016**) was a French boy, first child born to non-consanguineous parents with no familial history. Pregnancy was uneventful and delivery occurred at 40 weeks with normal growth parameters (3070 g, 52 cm, 33.5 cm). The infant died at three days of age in a context of severe respiratory failure and refractory hypoxemia despite intensive neonatal care (intubation ventilation). Autopsy showed lung hypoplasia (LW/BW=0.008), associated with bronchial cartilage dystrophy, disorganization of pulmonary parenchyma architecture, and rare distal aerial structure. No extra-pulmonary feature was noticed. Histological review was more in favor of congenital alveolar dysplasia (CAD).

Subject P016 was the younger sister of **subject P015**. Lung hypoplasia was suspected during pregnancy, as early as 22 weeks by ultrasounds, and then confirmed by fetal magnetic resonance imaging (MRI) at 29 weeks. Parents decided to continue the pregnancy and accompany the child at birth in palliative care if necessary. She was born at 39 weeks with normal growth parameters (3061 g, 50.5 cm, 34.2 cm) and she did indeed have major respiratory distress and died at 1 hour of life with comfort care, and without intensive resuscitation. Autopsy confirmed lung hypoplasia (LW/BW=0.009); no alveolar or saccular structure was identifiable. No extra-pulmonary feature was observed. Histological review concluded AcDys spectrum.

Subject P019 was the first child to unrelated Caucasian parents. He was born at term by natural vaginal delivery with mild evidence of fetal distress during 2nd stage of labor, and weight 3.5 kg. He developed severe hypoxia and respiratory distress in the minutes after birth requiring resuscitation, transfer from a peripheral hospital to tertiary center and escalated through inhaled nitric oxide, high frequency oscillatory ventilation (HFOV) and onto veno-arterial extracorporal membrane oxygenation (V-A ECMO) by about 12 hours of age. He was managed on ECMO for 10 days and was slowly weaned from ECMO, stabilized on continuous positive airway pressure mask (CPAP) and after a few days transitioned to Hi Flo nasal cannula oxygen delivery at an FiO2 at about 30%. At this stage many of his chest x-rays were remarkably clear. He remained tachypnoeic with minimal disturbance producing increased respiratory effort. Despite these suggestions of progress his pulmonary artery pressures remained suprasystemic (based on serial measures of tricuspid regurgitant jet or R to L shunt at the level of a small but persistently patent PDA). Sildenafil enabled weaning from inhaled nitric oxide, Bosentan in therapeutic doses seemed to have little additional benefit and was stopped but Prostacyclin at 15ng via surgically placed Hickman did on occasions appear to result in a reduction of PAP to 2/3 systemic. A lung biopsy was done and has been suggested to show some abnormalities of capillaries but not venous misalignment classical for alveolar capillary dysplasia (ACD).

Subject P022 was the first baby to an unrelated Caucasian couple. He was delivered by lower segment Caesarean section at term after an uneventful pregnancy. He had an initial weak cry and developed immediate respiratory distress. He was intubated but failed to improve. He developed a tension pneumothorax and required bilateral intercostal chest drains. Despite maximal efforts he became asystolic at 2 hours of age. Chest x-ray showed bilateral lung opacity, normal skeletal X-rays. Histological review concluded AcDys. The clinical history of **Subject P025** was described.[1]

Subject P026 was born at 40 weeks gestation to a 26 year old G3P1021 mother via vaginal delivery after labor induction. The pregnancy was uncomplicated except for maternal mitral prolapse, for which she received antibiotics during labor. Nuchal cord x 2 was present at delivery. The infant was notably cyanotic at birth with bradycardia and no respirations; Apgar scores 3 at one minute and 5 at 5 minutes. Birth weight 4000 g (94.2 percentile), length 54 cm (99.5 percentile), and head circumference 33.5 cm (37.4 percentile). She was intubated and received escalating positive pressure ventilation support resulting in a right-sided pneumothorax; two chest tubes were placed. Echocardiogram demonstrated a small muscular ventricular septal defect, suprasystemic pulmonary pressures with right to left intraductal shunt, and pulmonary artery branch hypoplasia. She was placed on veno-venous (V-V) ECMO for severe recalcitrant hypoxemia and combined respiratory and metabolic acidosis. She failed to recover and expired at

1 day of life. Autopsy examination demonstrated AcDys, a dilated pulmonary trunk with hypoplastic pulmonary artery branches and a right-sided aortic arch with vascular ring.

Subject P027 (sibling of **subject P035**) was born at 36 weeks gestation to a 45 year old G3P2 mother via cesarean section for variable biophysical profiles and prior poor pregnancy outcome. Prenatal ultrasounds demonstrated mild progressive growth restriction, a thickened placenta with venous lakes and an umbilical vein varix. The infant had respiratory distress at birth and was resuscitated vigorously with no response; he died at 5 hours of life. Postmortem examination demonstrated severe arrest of lung maturation in the spectrum of acinar dysplasia as well as ocular hypertelorism, accessory spleens (4) and massive perivillous fibrin deposition within the placenta. Histological review concluded AcDys with LW/BW=0.010.

Subject P028 was born at 38 weeks gestation to a 27 year old G6P3 mother via vaginal delivery after labor induction for chronic hypertension, smoking and recurrent herpes. During delivery there was decreased variability with late decelerations and a nuchal cord was present at delivery. The infant was cyanotic at birth which did not improve with positive pressure ventilation and was intubated. Apgar scores 4 at one minute, 6 at 5 minutes, birth weight 3459 g (60.2 percentile). An echocardiogram demonstrated right to left shunting at the patent ductus arteriosus and diffuse small branch pulmonary arteries. She failed to have a sustained response to high frequency oscillatory ventilation and inhaled nitric oxide and was placed on V-V ECMO. Due to her poor prognosis comfort measures were instituted and she expired at 4 days of life. Autopsy examination demonstrated AcDys and a dilated pulmonary trunk with small caliber of pulmonary artery branches.

Subject P033 was born at 40 weeks gestation via induced vaginal delivery due to high blood pressure in her mother. Prenatal screening included a combined screen and anatomy ultrasound, both of which were normal. Birth parameters included a weight of 3287 g (27th percentile), length of 51 cm (46th percentile), and OFC of 33.5 cm (14th percentile). Her initial 1 minute Apgar score was 8, and she was place on her mother's chest. At 3 minutes of life she became apneic and cyanotic. Bag mask ventilation was initiated and she was intubated at 11 minutes of life. Subsequently, she was admitted to the NICU with rapid escalation of respiratory support for significant hypoxemia. On chest x-ray she was noted to have bilateral pneumothoraces and required needle decompression followed by bilateral chest tubes; however, she remained hypoxemia. She was placed on high-frequency ventilation with mean airway pressures titrated from 16 to 26 without any improvement in oxygenation. She was transferred to a tertiary care center, and attempts were made to maximize her settings with nitric oxide, dopamine infusion, and epinephrine. During that time, she was noted to have hemothoraces and was transfused with 10ml/kg of packed red blood cells, cryoprecipitate, and fresh frozen plasma (FFP). Diagnostic echocardiogram showed a large unrestrictive patent ductus arteriosus with right to left shunting, branch pulmonary arteries appeared subjectively small, but was otherwise normal. Cranial ultrasound was negative for bleeding. Thus, given persistent respiratory failure and cardiopulmonary instability requiring one round of CPR, she was placed on V-A ECMO. On physical exam, she was nondysmorphic in appearance. Given her critical status, rapid in subject exome sequencing and lung biopsy was performed to evaluate for congenital lung dysplasia. At 3 days of life, lung biopsy pathology was consistent with AcDys of the lungs. Given the lethality of the disease her care was redirected to comfort measures at that time. Autopsy examination demonstrated LW/BW=0.008 and confirmed AcDys. Family history includes Caucasian ancestry

and no known consanguinity. On the maternal side, her mother and maternal grandfather both have Crohn's disease. Her maternal grandmother has asthma. On the paternal side, her father was born without functioning tear ducts and has dental abnormalities. Her paternal grandfather, paternal uncle and cousin have absent tear ducts and/or asthma. Her maternal grandmother has Sjogren's disease. The family history was otherwise negative for known genetic syndromes, childhood deaths, developmental delays, lung disease, birth defects, or recurrent miscarriages.

Subject P034 (sibling of **subject P027**) was born at 32 weeks gestation to a 41 year old G1P1 mother via Cesarean section for absent end diastolic flow. Pregnancy complicated by intrauterine growth restriction, early oligohydramnios, bilateral lung cysts (detected at 29 weeks gestation and stable on subsequent scans), mild cardiomegaly and a posteriorly thickened placenta; normal 46,XX on amniocentesis. She developed respiratory failure shortly after birth and required high-frequency oscillatory ventilation and pressors. Echocardiogram demonstrated findings of pulmonary hypertension with significant right-to-left shunting. She was transitioned to palliative measures and died at 21 hours of life. Autopsy examination demonstrated a profound arrest in lung maturation suggestive of CAD versus pulmonary hypoplasia. Superimposed diffuse alveolar damage was present. There were facial features and limb deformations consistent with prolonged oligohydramnios. The placenta showed massive perivillous fibrin deposition.

Subject P035 was born at 33 weeks gestation to a 40 year old G3P2 mother via Cesarean section for decreased fetal movement and bradycardia. Pregnancy complicated by intrauterine growth restriction, thrombophilia (on Lovenox) and thyroid cancer s/p thyroidectomy. Prenatal screening included normal cell-free fetal DNA. Birth weight 1800 g (13th percentile), length of 45 cm (57th percentile), and head circumference 28.5 cm (6th percentile). Apgar scores 5 at one minute, 5 at 5 minutes, and 8 at 10 minutes. He required rapid escalation in support shortly after delivery including intubation and pressors; developed bilateral tension pneumothoraces s/p chest tubes. He was placed on V-A ECMO for refractory hypoxemia and hypotension. Echocardiogram demonstrated findings of pulmonary hypertension and iNOS was started. Dysmorphic features included bilateral clenched fists, mild low-set, posteriorly rotated ears, widely spaced nipples, broad first toes with hypoplastic toenails and bilateral 2^{nd} toe clinodactyly. A cord-blood karyotype demonstrated normal 46,XY. Given his critical status, rapid exome sequencing and lung biopsy were performed on day 8, the latter consistent with a lethal lung dysplasia. His care was redirected to comfort measures and he died at day 10. Autopsy examination confirmed arrested lung development in the spectrum of AcDys as well as right ventricular hypertrophy and above detailed dysmorphic features.

Subject P038 was born at 35^{+6} weeks' gestation with a birth weight of 1900 g and Apgar scores of 4 at 1 minute, 6 at 5 minutes, and 6 at 10 minutes. The arterial cord pH was 7.34 with a lactate of 3. He cried at birth and was given CPAP and mask intermittent positive pressure ventilation (IPPV) for cyanosis. He remained hypoxic with oxygen saturations in the low 40's despite high pressures of mask IPPV; chest movement remained poor. He was intubated and ventilated, and his oxygen saturations increased to the 50's. He was transferred to NICU at 35 minutes of age. The cervical kyphosis was not evident at birth. His clinical course was consistent with a diagnosis of hypoplastic lungs and he was managed with mechanical ventilation, including high frequency oscillatory, and nitric oxide. He developed a right pneumothorax and a mild pneumomediastinum, the former of which was treated with a right-sided intercostal chest drain that was inserted at 2 hours of age. His oxygenation did not improve on nitric oxide or on re-

expansion of his lungs. He was ventilated at high pressures but deteriorated at 8 hours of age with worsening hypoxia and acidosis despite full support. He was extubated at 4.20 hrs and died peacefully in his parents' arms at 4.40 hrs, at just over 12 hours of age. A complete autopsy revealed a small-for-gestational age male infant with a birth weight below the 10th centile. There were no external dysmorphic features. The lungs were hypoplastic (based on a reduced combined LW/BW ratio of 0.009), with subsequent histological examination revealing the diagnosis of CAD. The long bones were short (their measurements being average for around 30-32 weeks' gestation), but there were no other significant skeletal abnormalities with no evidence of skeletal dysplasia. There was unilateral left renal agenesis, while the right kidney showed mild pelvicalyceal dilation and mild hydroureter, possibly secondary to vesicoureteral reflux; there was no renal dysplasia and no evidence of posterior urethral valves. There was mild ventricular disproportion of the heart, the right ventricle being larger than the left, but the heart was otherwise structurally normal.

Subject P040 was a girl who was born by Cesarean at 34w3d gestation for worsening fetal growth restriction and abnormal fetal monitoring. The pregnancy had been complicated by fetal pericardial effusion and small aortic valve seen on ultrasound. Amniocentesis revealed a deletion consistent with LADD syndrome, found to be maternally inherited. Apgar scores were 8, 8 and birth weight was 2090 g. The girl was intubated for respiratory distress and treated with surfactant. She could not be adequately oxygenated by any method of mechanical ventilation. No anatomic cause for this was apparent, though on cardiac echo the branch pulmonary arteries appeared small. She was placed on ECMO but she developed bilateral grade II-III intraventricular hemorrhages with intracranial hypertension, systemic hypertension, and bradycardia, and she died at 5 days of age. Based on autopsy weights, the LW/BW for that child was 0.009, well below the normal range, and the lungs were abnormal on microscopic evaluation indicating pulmonary hypoplasia. She had a broad forehead, but no other physical features for LADD syndrome were apparent.

Subject P041 was a 2720 gram female infant delivered by repeat elective C-section to a 34 year old gravida 3, para 4. Prenatal ultrasound had shown no abnormalities. Apgar scores were 6, 9. Soon after birth the baby developed grunting and cyanosis. Initial x-rays showed a small right pneumothorax and poor expansion of the left lung. Repeat chest x-ray two hours after birth showed persistent right pneumothorax. Following placement of a chest tube, the infant had worsening hypoxia. She was intubated and hand bagged with no improvement; x-ray showed only minimal aeration of the right lung, and little lung expansion on the left. The infant died four hours and twenty minutes after birth. At autopsy both lungs showed no evidence of aeration. The left and right lungs showed generalized hypoplasia, with a combined weight of 25 g (LW/BW ratio 0.009, well below the normal range for any gestational age).

Subject P042 was the first girl born to non-consanguineous French parents with no familial history. She had then 2 healthy siblings. Pregnancy was uneventful, and delivery occurred at 41 weeks by emergency cesarean section due to fetal bradycardia. Birth parameters were normal (3235 g, 51 cm, 32.5 cm). The Apgar score was initially 10 but then deteriorated very quickly. She was intubated at 15 minutes of life but HFOV did not provide sufficient oxygen saturation nor the addition of nitric oxide and surfactant. Cardiac echography showed pulmonary hypertension but no cardiac malformation. She died at 10 hours of life. Autopsy showed lung

hypoplasia (LW/BW=0.008), apparent arrest of pulmonary maturation at late canalicular stage, dysplastic cartilage, and severe congestion. No extra-pulmonary feature was noticed.

Subject P043 was a boy, first child born to non-consanguineous French parents with no familial history. He had then a healthy sister. Pregnancy was uneventful, and delivery occurred at 37 weeks with mild IUGR (2550 g, 48 cm, 30.5 cm). He had immediate respiratory distress with severe pulmonary hypertension, and received intensive resuscitative care, with the implementation of an extracorporeal membrane oxygenation. A lung biopsy was performed at 5 days of life and showed a poorly developed lung with great immaturity at canalicular or first saccular stages. Assessment was hampered by ventilation superimposed injury. Histological review concluded AcDys. No extra-pulmonary associated feature was observed. He died at 15 days of life. No autopsy was performed.

Subject P044 was the first case described in the publication[2]. They reported on two Belgian sisters who died neonatally from severe pulmonary hypoplasia. The first girl was born at 40 weeks, after an uneventful pregnancy, with mild IUGR (2860 g, 49.5 cm, 32.5 cm). She developed severe respiratory distress immediately after birth, and died at two days of life despite active intensive treatment. Autopsy showed lung hypoplasia (LW/BW=0.007), with reduced alveolar parenchyma, complete absence of mature alveoli, increased amount of interstitial connective tissue, and dysplastic bronchial cartilage plates. Maturation stopped probably at early canalicular stage. No histological review could have been done.

Subject P045 was a female fetus, from unrelated French parents with no familial history. It was initially a twin pregnancy but the other twin died in utero at 16 weeks. Severe lung hypoplasia was identified at 22 weeks in the remaining fetus, confirmed with a fetal MRI at 33 weeks. The pregnancy has been arrested at 38 weeks. Fetopathological examination confirmed lung hypoplasia (LW/BW=0.006), associated with marked dysplastic cartilage and an arrest of maturation at pseudoglandular stage. Histological review concluded AcDys. Hypertrophic ovaries were also noticed.

Subject P046 was a boy, born to non-consanguineous French parents with no familial history. Pregnancy and delivery were uneventful. He developed immediately respiratory distress and died at 8 hours of life despite intensive neonatal care. Autopsy showed lung hypoplasia and complete arrest of pulmonary maturation at pseudoglandular stage. Histological review concluded AcDys. No extra-pulmonary feature was noticed.

Subject P048 was a girl, third child of parents with known consanguinity. The first child was a boy, who died at 3 hours of life in a context of severe respiratory failure. The second fetus died in utero at 37.5 weeks, with lung hypoplasia. For this third pregnancy, lung hypoplasia was diagnosed at 22 weeks and a prenatal treatment by plug was performed. Delivery occurred at 33 weeks with normal growth parameters (2116 g, 50 cm, 30 cm), and the baby died after 50 minutes of life. Autopsy showed mild lung hypoplasia (LW/BW=0.014), with a stop of maturation at late canalicular to saccular stage. These pulmonary abnormalities were associated with coarse facial features. Histological review concluded pulmonary hypoplasia.

Subject P073 was a girl, third child of unrelated French parents. The two first siblings are healthy. Pregnancy was uneventful and delivery occurred at 40 weeks with normal growth parameters (3635 g, 49 cm, 32.5 cm), with Apgar score at 10. Then she developed quickly

respiratory distress with refractory hypoxemia and severe pulmonary hypertension, and was intubated at 40 minutes of life. She died within the firsts 24 hours despite active neonatal care. Autopsy revealed lung hypoplasia (LW/BW=0.006), with the absence of alveoli and a stop of pulmonary maturation at saccular stage. No extra-pulmonary feature was noticed. No histological review could have been done.

Subject P076 was the baby girl who died 5 days after birth due to severe congenital hypoplasia of the lungs. Dysmorphologic examination at the time was difficult because of the serious lung problems. The external ears were a bit dysplastic with mild over-folding of the helix. There was no polydactyly. The father was diagnosed with LADD syndrome in the past. Autopsy revealed pulmonary hypoplasia.

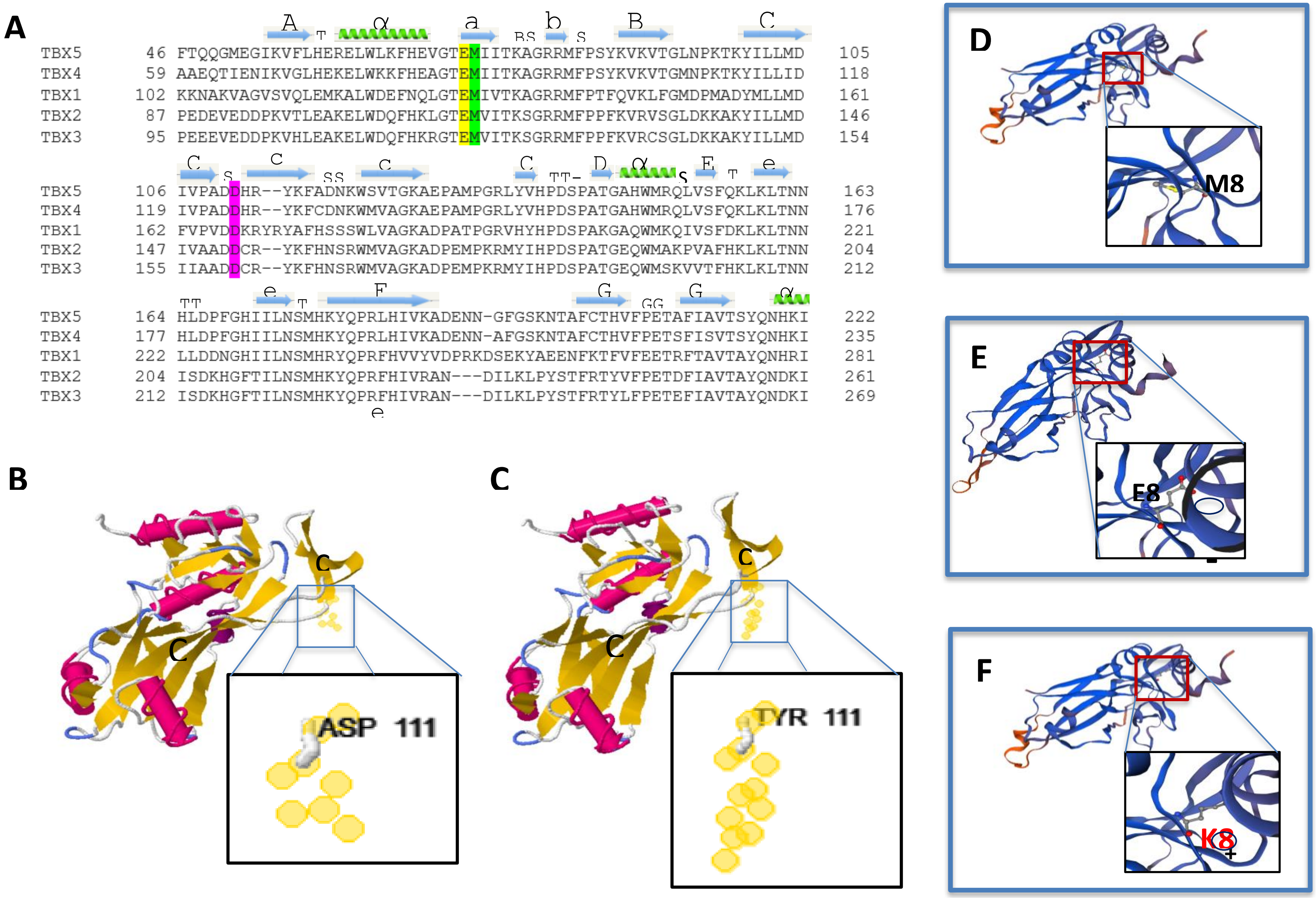

A
TBX5 46 FTQQGMEGIKVFLHERELWLKFHEVGTEMIITKAGRRMFPSYKVKVTGLNPKTKYILLMD 105
TBX4 59 AAEQTIENIKVGLHEKELWKKFHEAGTEMIITKAGRRMFPSYKVKVTGMNPKTKYILLID 118
TBX1 102 KKNAKVAGVSVQLEMKALWDEFNQLGTEMIVTKAGRRMFPTFQVKLFGMDPMADYMLLMD 161
TBX2 87 PEDEVEDDPKVTLEAKELWDQFHKLGTEMVITKSGRRMFPPFKVRVSGLDKKAKYILLMD 146
TBX3 95 PEEEVEDDPKVHLEAKELWDQFHKRGTEMVITKSGRRMFPPFKVRCSGLDKKAKYILLMD 154
TBX5 106 IVPADDHR--YKFADNKWSVTGKAEPAMPGRLYVHPDSPATGAHWMRQLVSFQKLKLTNN 163
TBX4 119 IVPADDHR--YKFCDNKWMVAGKAEPAMPGRLYVHPDSPATGAHWMRQLVSFQKLKLTNN 176
TBX1 162 FVPVDDKRYRYAFHSSSWLVAGKADPATPGRVHYHPDSPAKGAQWMKQIVSFDKLKLTNN 221
TBX2 147 IVAADDCR--YKFHNSRWMVAGKADPEMPKRMYIHPDSPATGEQWMAKPVAFHKLKLTNN 204
TBX3 155 IIAADDCR--YKFHNSRWMVAGKADPEMPKRMYIHPDSPATGEQWMSKVVTFHKLKLTNN 212
TBX5 164 HLDPFGHIILNSMHKYQPRLHIVKADENN-GFGSKNTAFCTHVFPETAFIAVTSYQNHKI 222
TBX4 177 HLDPFGHIILNSMHKYQPRLHIVKADENN-AFGSKNTAFCTHVFPETSFISVTSYQNHKI 235
TBX1 222 LLDDNGHIILNSMHRYQPRFHVVYVDPRKDSEKYAEENFKTFVFEETRFTAVTAYQNHRI 281
TBX2 204 ISDKHGFTILNSMHKYQPRFHIVRAN---DILKLPYSTFRTYVFPETDFIAVTAYQNDKI 261
TBX3 212 ISDKHGFTILNSMHKYQPRFHIVRAN---DILKLPYSTFRTYLFPETEFIAVTAYQNDKI 269
B
C
C
ASP 111
C
C
C
TYR 111
D
M8
E
E8
F
K8

Figure S1. Potential consequences of the p.E86K variant in TBX4 and the p.D111Y variant in TBX5 on the T-BOX function. (**A**) ClustalW sequence alignment of the T-BOX domains of the human TBX1-5 proteins equivalent to the TBX5 residues 46-222. Secondary structural elements are represented by blue arrows for β-strands and green zigzag ribbons for the α-helices. S=bend. B=residue isolated in β bridges. G=3-turn helix. T=hydrogen bonded turn. Residues of TBX4 and TBX5 mutated in this study are highlighted in yellow and magenta. The highly conserved residue E86 of TBX4 (yellow) corresponds to the E73 residue of TBX5 adjacent to the previously characterized M74 (green). The highly conserved residue D111 of TBX5 (magenta) and is predicted to be involved in β-turn forming which is likely to be disrupted by the Tyr (Y) substitution (see B and C). (**B**) 3D simulation of the TBX5 T-BOX based on the crystal structure of human TBX5 (PDB_ c5flvA_) obtained with the Phyre2 bioinformatic tool (http://www.sbg.bio.ic.ac.uk/phyre2). Alpha-helices are shown as rockets in red, beta-strands as yellow ribbons. The highly conserved D111 in located on the turn between C and c β-strands. (**C**) The substitution D111Y could affect the 3D conformation of the T-box domain by changing the β -forming residue Asp to non β -forming Tyr residue. (**D**) 3D simulation of TBX4 T-box domain based on human TBX5 structure (PDB_ c5flvA_) performed by the Swiss-Model showing the location of the key residue M87 corresponding to the previously reported M74 in TBX5 which lies next to the E86 mutated in our study. The non-conservative change E86K inverts the polarity from negative (**E**) to positive (**F**) and thus might affect the stabilization of the hydrophobic core close to the active site compromising binding ability of TBX4.

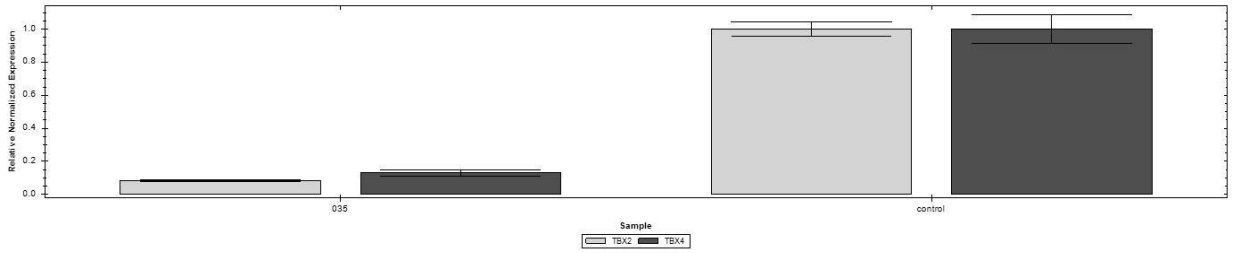


Figure S2. Comparative RT qPCR analysis of the *TBX2* and *TBX4* mRNA levels in lung tissues. Comparison of expression levels of *TBX2* and *TBX4* in lung tissue of affected subject P035 with 17q23 deletion. Normal lung tissue was used as a negative control. Data are represented as the mean ± SEM.

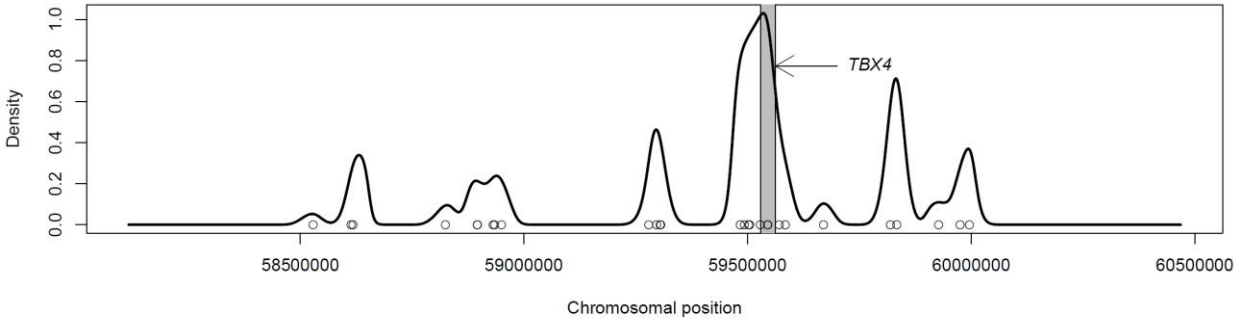


Figure S3. Distribution of SNPs analyzed using Affymetrix CytoScan HD SNP array. The graph represents distribution of SNPs analyzed using Affymetrix CytoScan HD SNP array in subjects (P006, P009, P012, P019, and P026) with the heterozygous 17q23.1q23.2 deletion and two subjects with heterozygous *TBX4* SNV (P022 and P025), and absent in the control individuals with the same deletion but without any structural lung abnormalities (C051, C054, C055, C058, and C059).

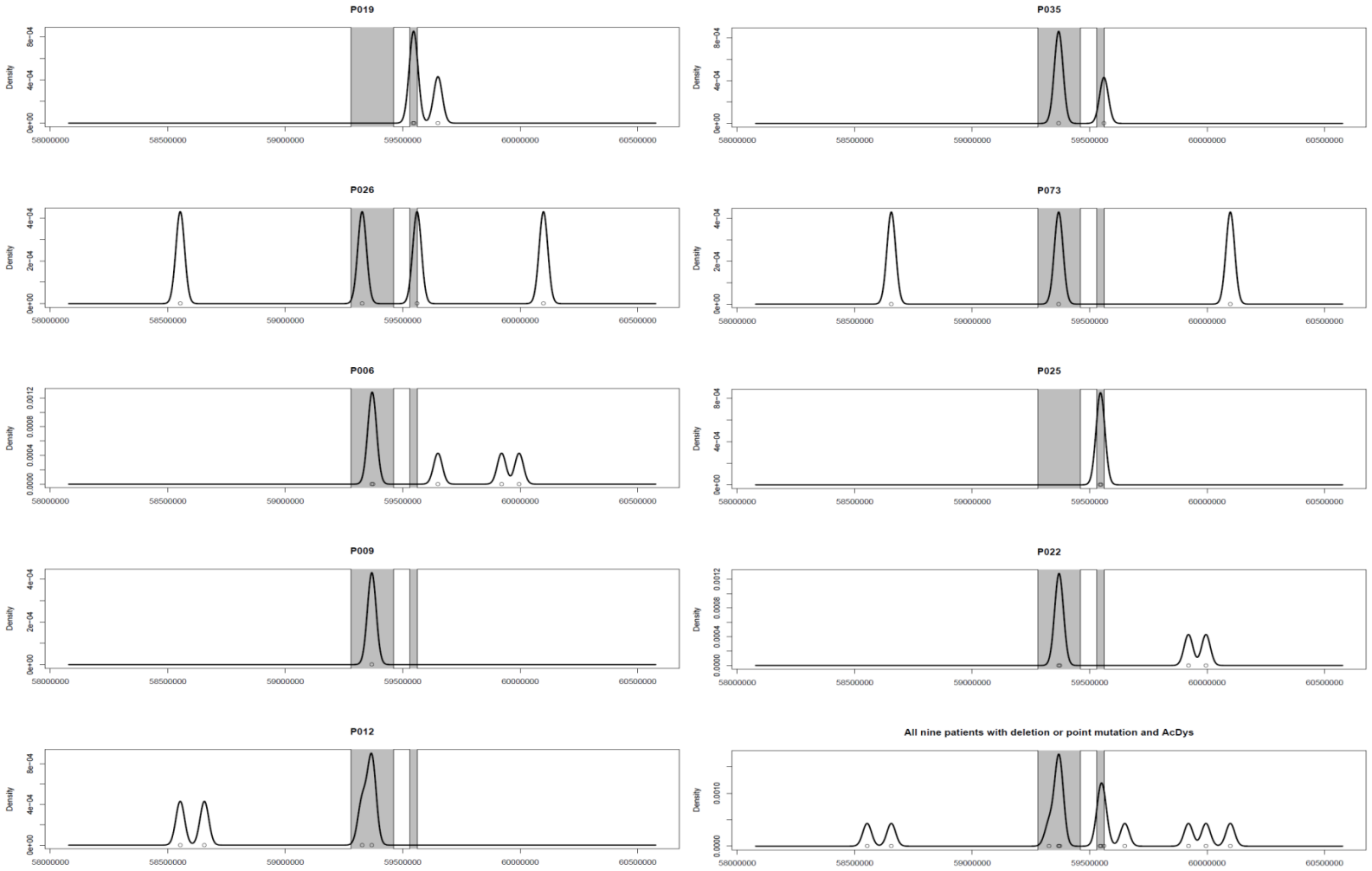


Figure S4. Distribution of the selected SNVs identified by WGS in the 17q23.1q23.2 deletion region showing their enrichment. In this analysis, we have considered variants with MAF < 10% (GnomAD, r2.0.2) that are shared by at least two affected individuals (P006, P009, P012, P019, P026, P035, P073) with 17q23.1q23.2 deletion and two subjects with heterozygous *TBX4* point mutation (P022 and P025) but absent in 13 control individuals with the same deletion but without any structural lung abnormalities.

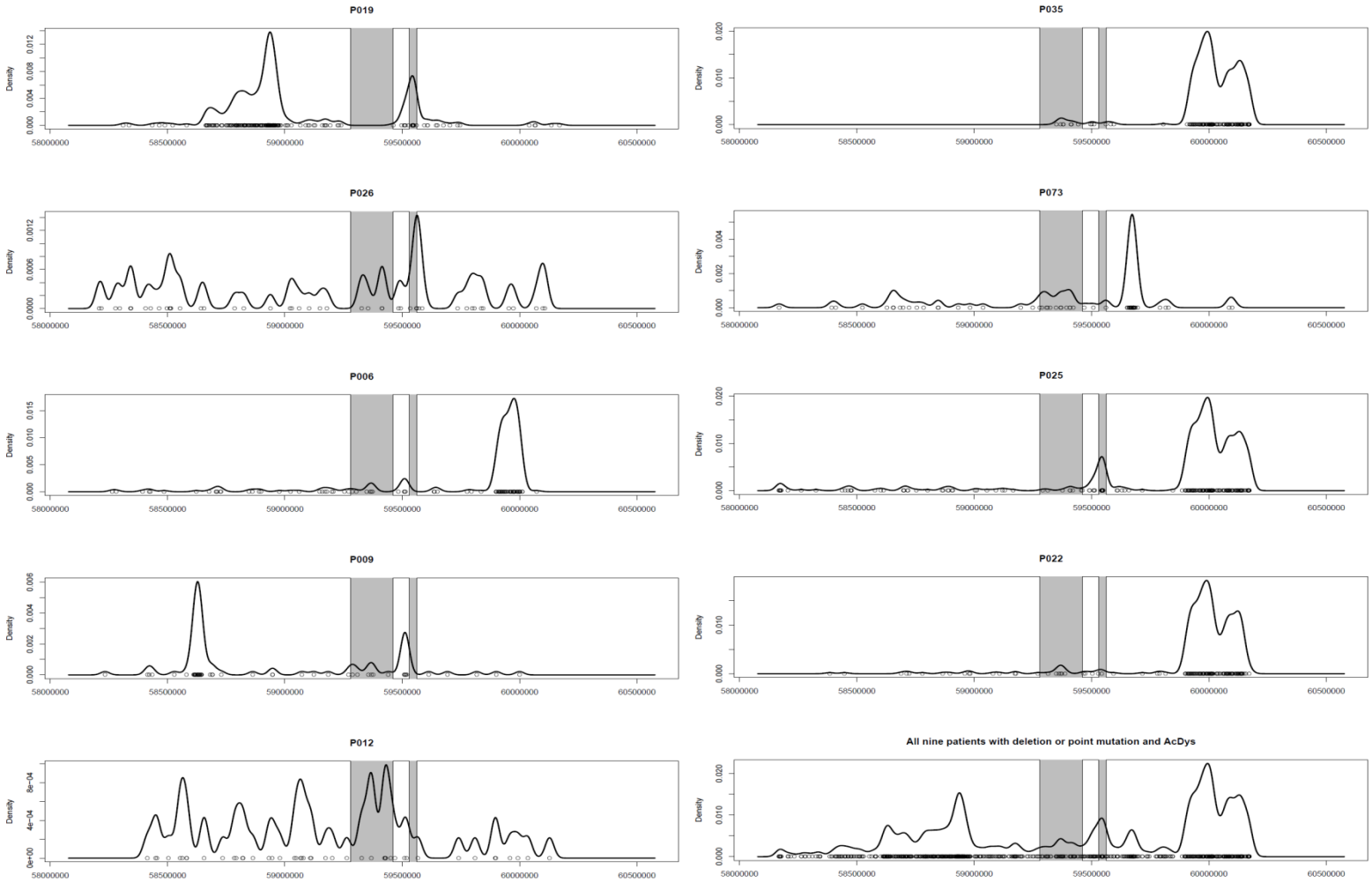
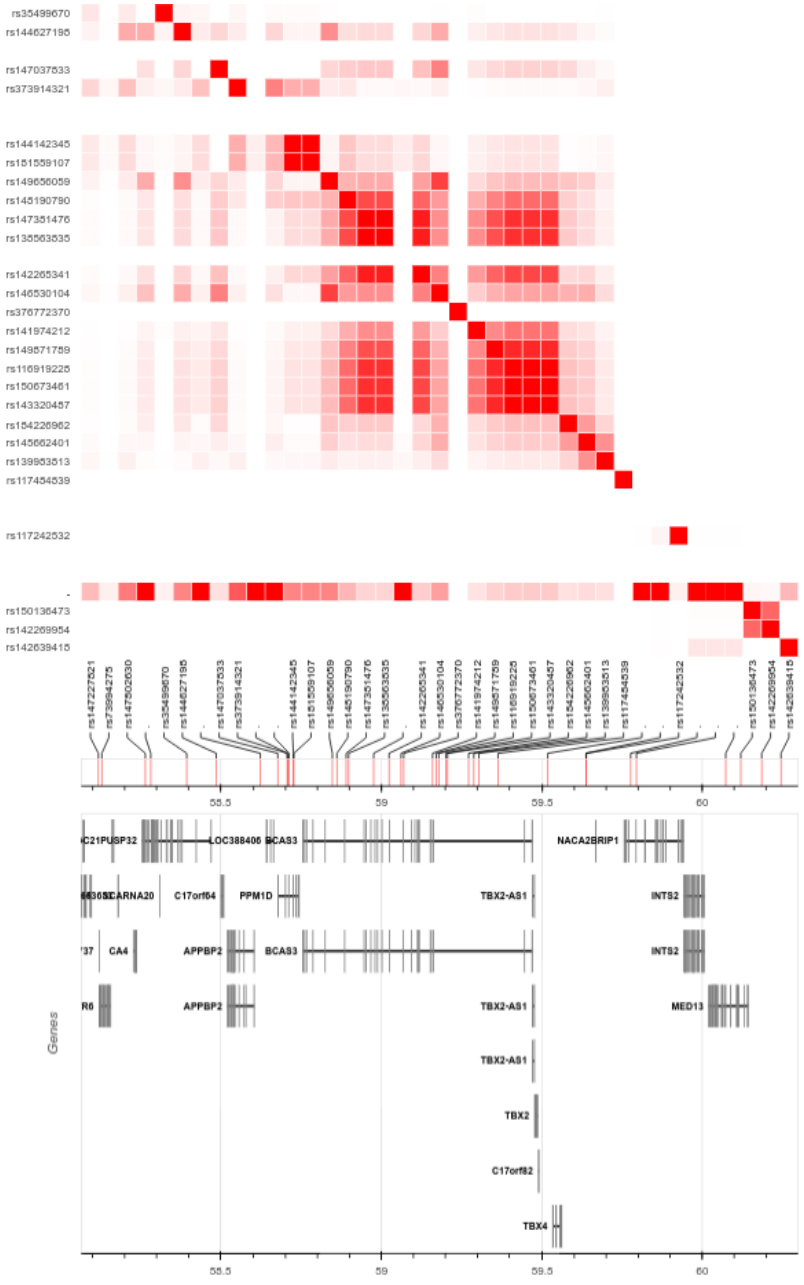
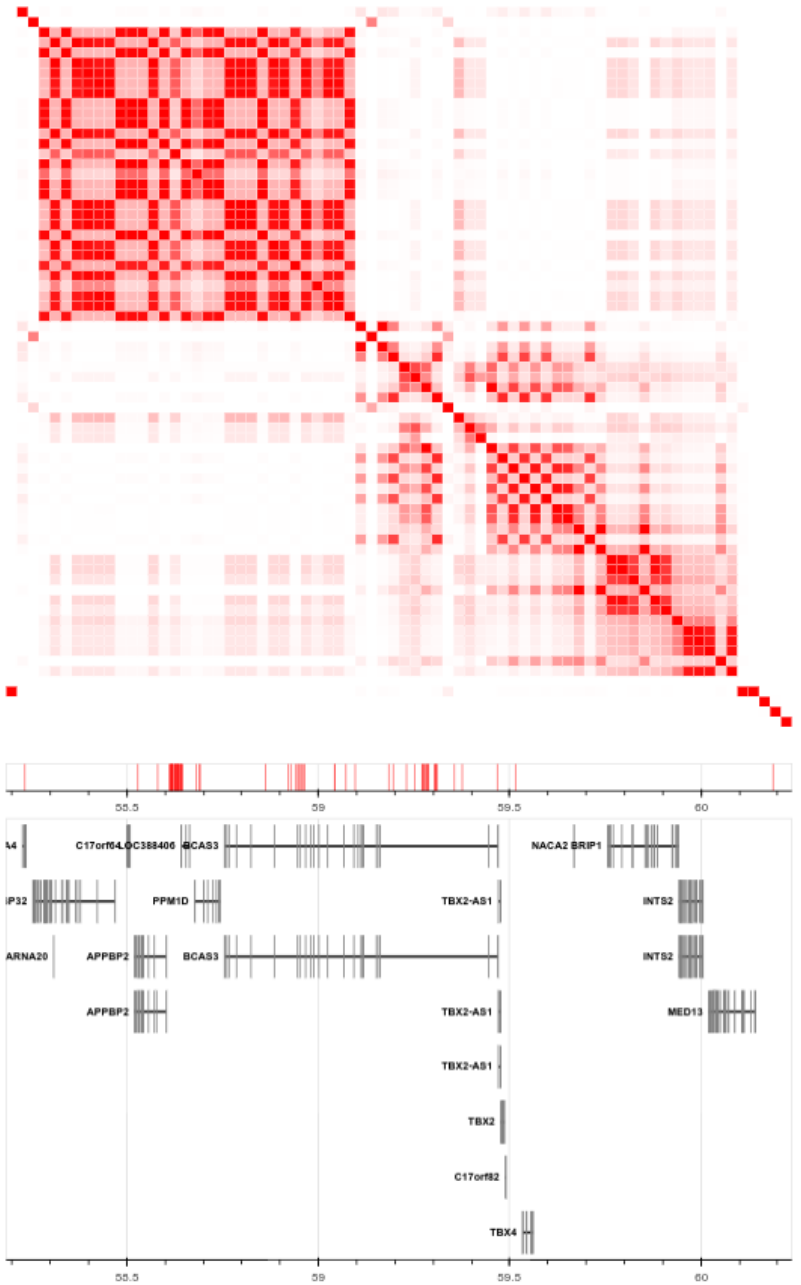


Figure S5. Distribution of all SNVs identified by WGS in the 17q23.1q23.2 deletion region. The graphs show the distribution of all SNVs identified by WGS in seven affected individuals with the heterozygous 17q23.1q23.2 deletion (P006, P009, P012, P019, P026, P035, P073) and two subjects with the heterozygous *TBX4* missense mutations (P022 and P025), absent in 13 control individuals with overlapping deletions.

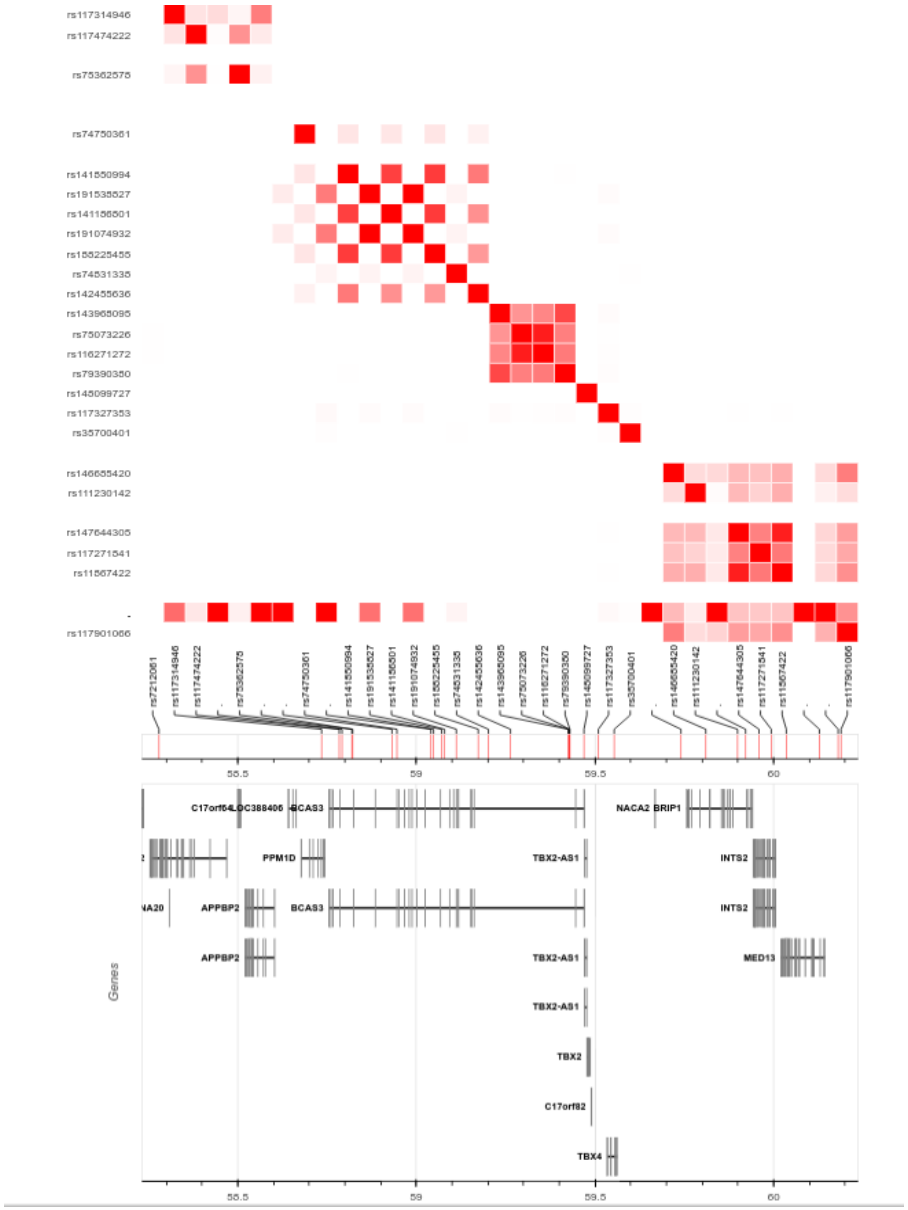
P006


rs35499670
rs144627195
rs147037533
rs373914321
rs144142345
rs151559107
rs149656059
rs145190790
rs147351476
rs135563535
rs142265341
rs146530104
rs376772370
rs141974212
rs149571759
rs116919225
rs150673461
rs143320457
rs154226962
rs145662401
rs139953513
rs117454539
rs117242532
rs150136473
rs142269954
rs142639415
55.5
59
59.5
60
C21PUSP32
LOC388406
BCAS3
NACA2BRIP1
ARNA20
C17orf64
PPM1D
TBX2-AS1
INTS2
CA4
APPBP2
MED13
TBX2
C17orf82
TBX4
Genes

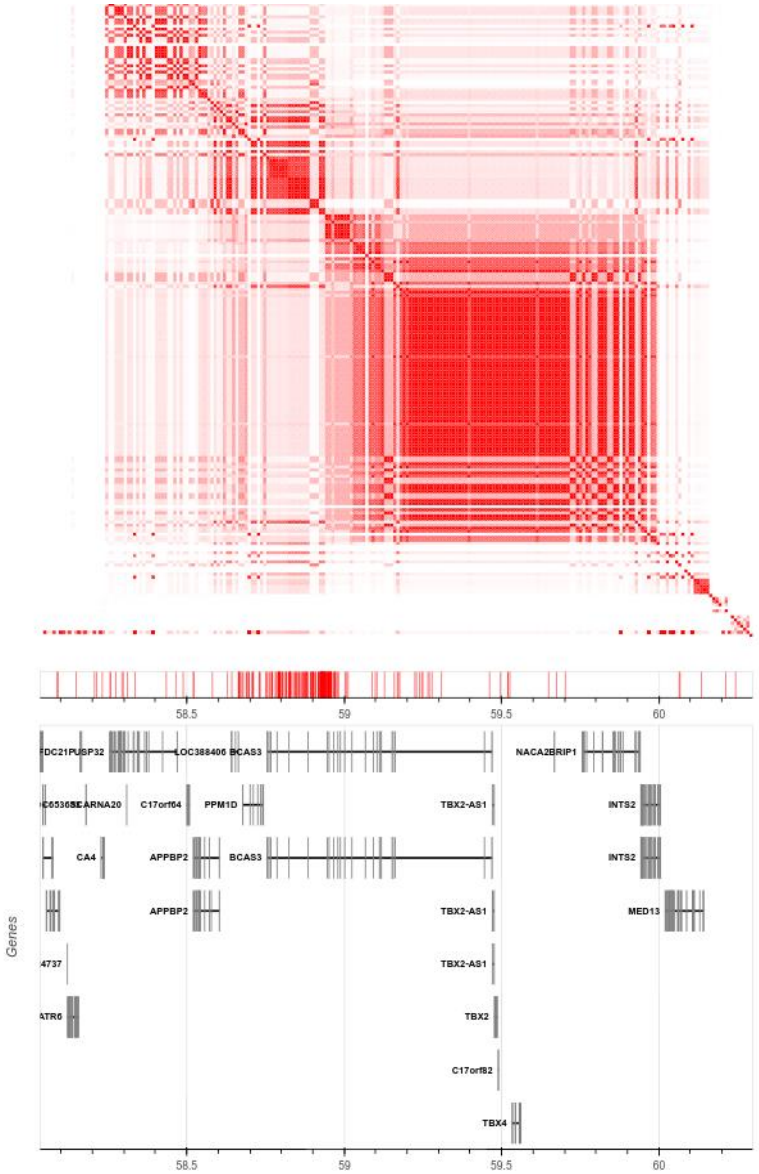
P009


55.5
59
59.5
60
C17orf64
LOC388406
BCAS3
NACA2BRIP1
PPM1D
TBX2-AS1
INTS2
ARNA20
APPBP2
MED13
TBX2
C17orf82
TBX4

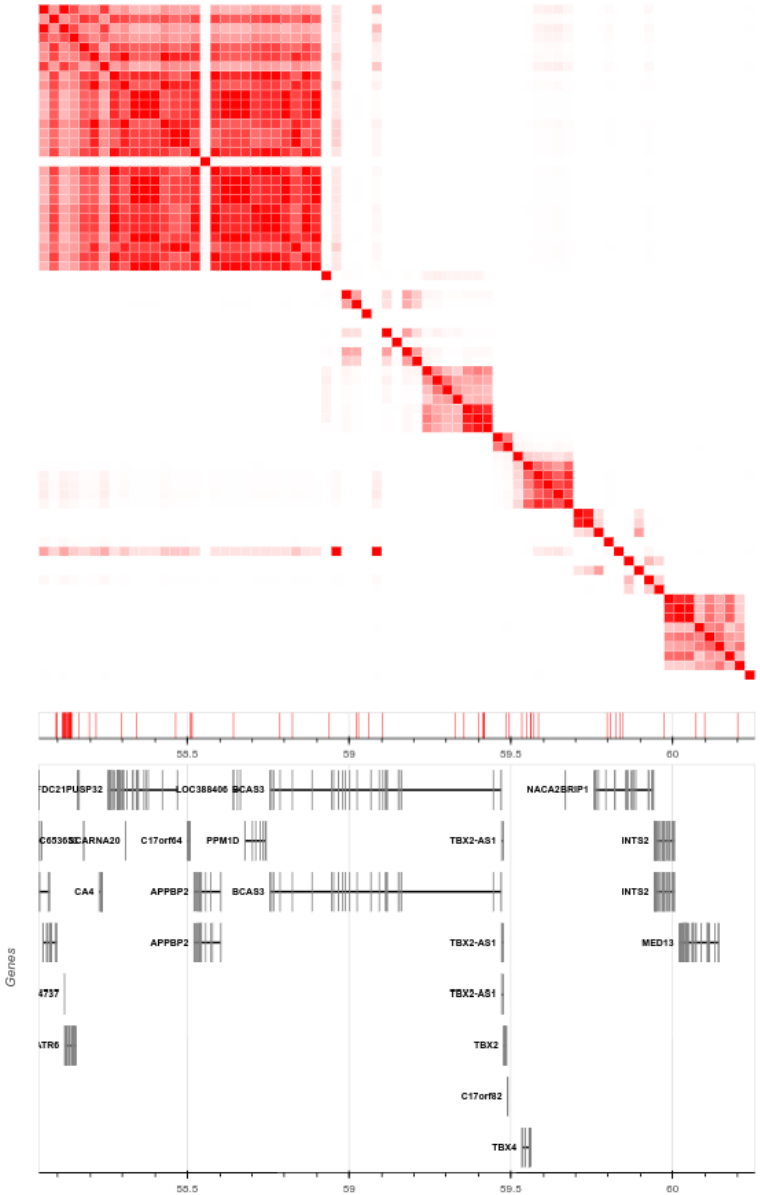
P012


rs117314946
rs117474222
rs75362575
rs74750361
rs141550994
rs191535527
rs141156501
rs191074932
rs155225455
rs74531335
rs142455636
rs143965095
rs75073226
rs116271272
rs79390350
rs145099727
rs117327353
rs35700401
rs146655420
rs111230142
rs147644305
rs117271541
rs11567422
rs117901066
58.5
59
59.5
60
C17orf64
LOC388406
BCAS3
NACA2
BRIP1
PPM1D
TBX2-AS1
INTS2
APPBP2
MED13
TBX2
C17orf82
TBX4
Genes

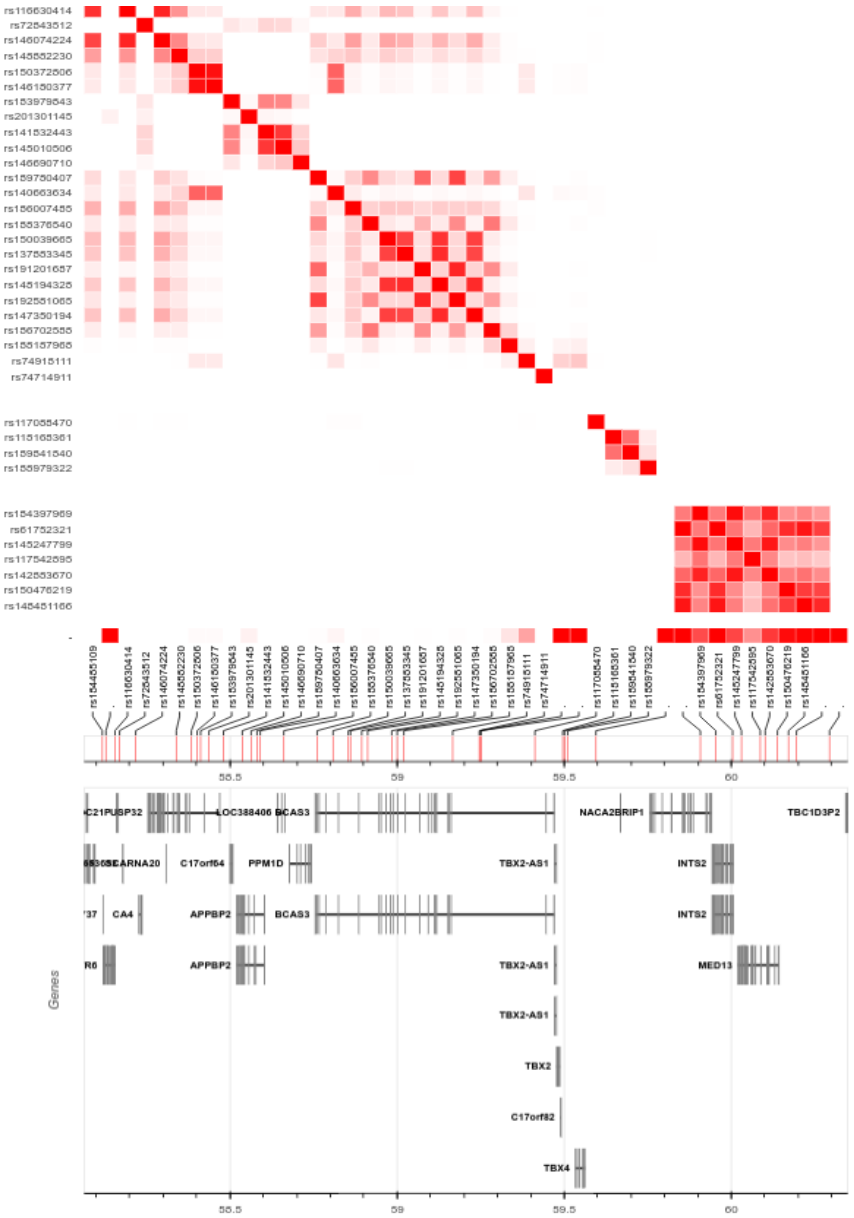
P019


58.5
59
59.5
60
LOC388406
BCAS3
NACA2
BRIP1
C17orf64
PPM1D
TBX2-AS1
INTS2
CA4
APPBP2
MED13
TBX2
C17orf82
TBX4
Genes

P026


55.5
59
59.5
60
Genes
NACA2BRIP1
TBX2-AS1
INTS2
INTS2
APPBP2
BCAS3
APPBP2
TBX2-AS1
MED13
TBX2-AS1
TBX2
C17orf82
TBX4

P035


rs116630414
rs72543512
rs146074224
rs145552230
rs150372506
rs146150377
rs153979543
rs201301145
rs141532443
rs145010506
rs146690710
rs159750407
rs140663634
rs156007455
rs155376540
rs150039665
rs137553345
rs191201657
rs145194325
rs192551065
rs147350194
rs156702555
rs155157965
rs74915111
rs74714911
rs117055470
rs115165361
rs159541540
rs155979322
rs154397969
rs61752321
rs145247799
rs117542595
rs142553670
rs150476219
rs145451166
55.5
59
59.5
60
Genes
NACA2BRIP1
TBC1D3P2
INTS2
INTS2
APPBP2
BCAS3
APPBP2
TBX2-AS1
MED13
TBX2-AS1
TBX2
C17orf82
TBX4

P073

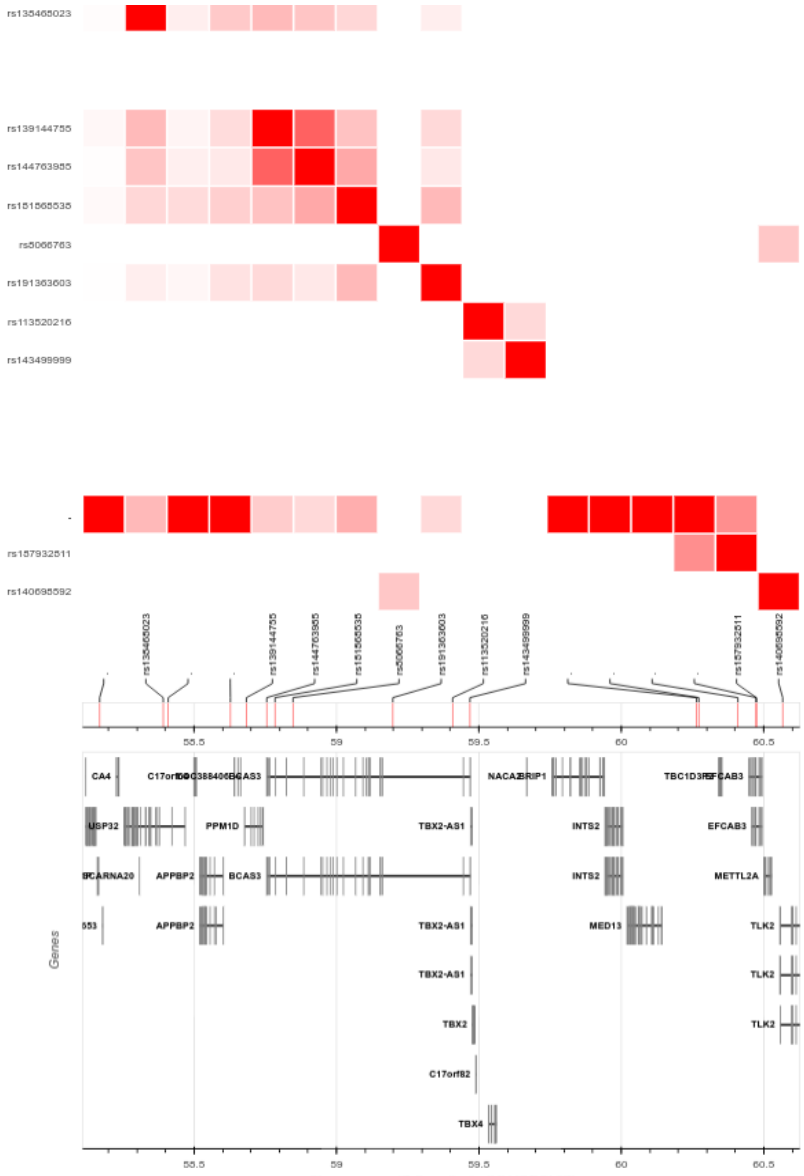


Figure S6. Haplotypes of affected individuals. The figures show haplotypes identified in affected individuals P006, P009, P012, P019, P026, P035, P073 with the heterozygous 17q23.1q23.2 deletion.

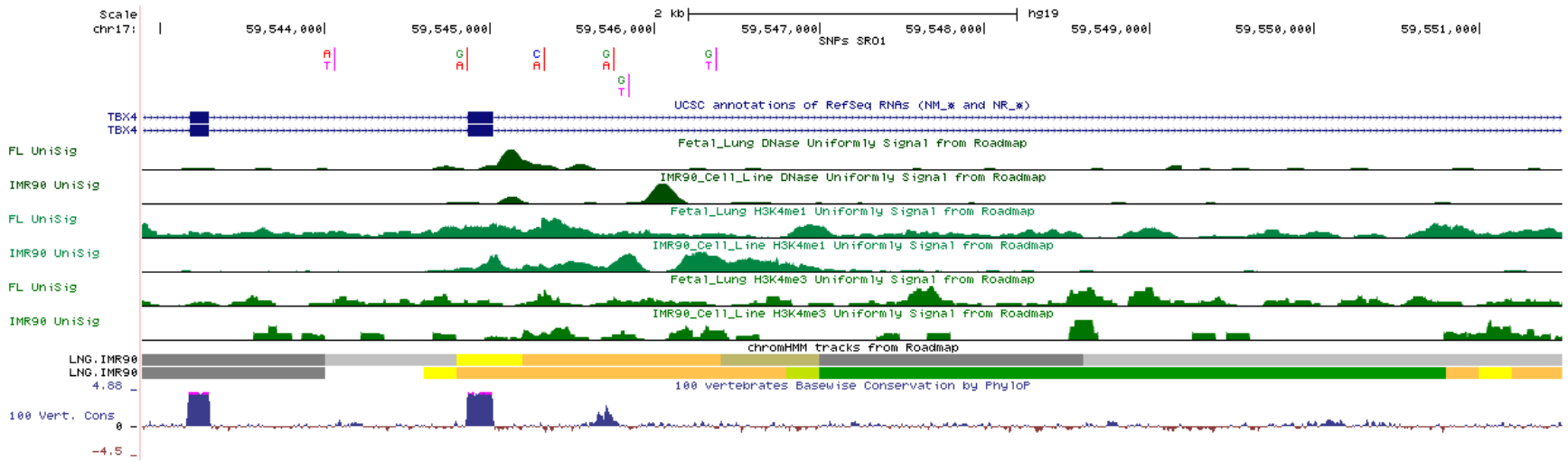


Figure S7. Variants identified in enhancer region located within *TBX4*. The graph includes DNaseI hypersensitivity clusters, H3KMe1, and H3KMe3 marks in the IMR-90 cell line and the fetal lung; chromatin state annotation is based on ChIP-seq mapping (Roadmap) in the IMR-90 cell line and conservation scores (PhyloP). The block of six SNPs identified within deletion region in *TBX4* in affected individuals is indicated above the *TBX4* gene.

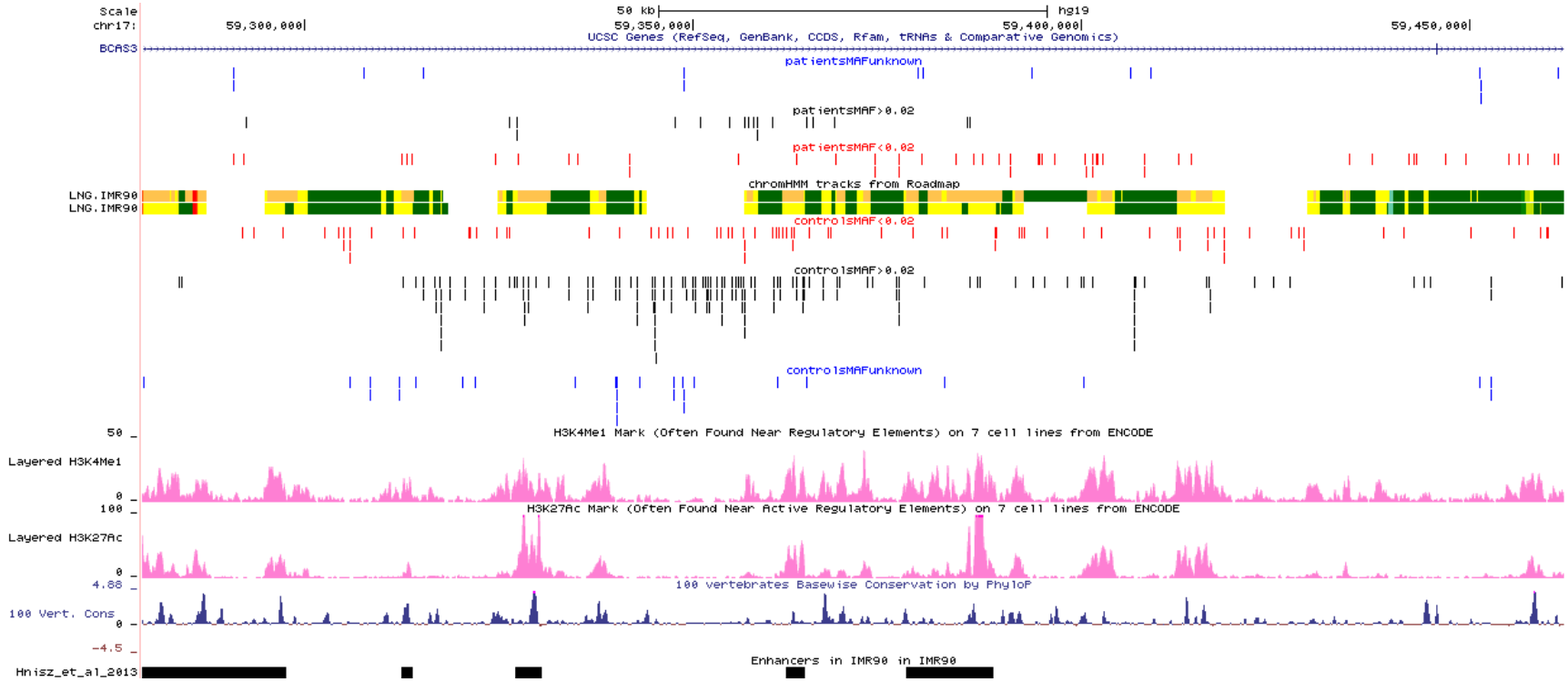


Figure S8. Schematic representation of the lung-specific enhancer region located upstream to *TBX4*. The graph represents H3KMe1 and H3KMe3 marks in the IMR-90 cell line and the fetal lung; chromatin state annotation is based on ChIP-seq mapping (Roadmap) in the IMR-90 cell line, conservation scores (PhyloP) and the enhancers identified in IMR-90 cell line. SNVs identified in affected individuals are presented in the top of chromatin state annotation block, while SNVs identified in the controls are shown below this track. SNVs with gnomAD (r2.0.2) MAF>=0.2 are shown in red; SNVs with MAF>0.2 are shown in black, and SNVs with unknown MAF are shown in blue.

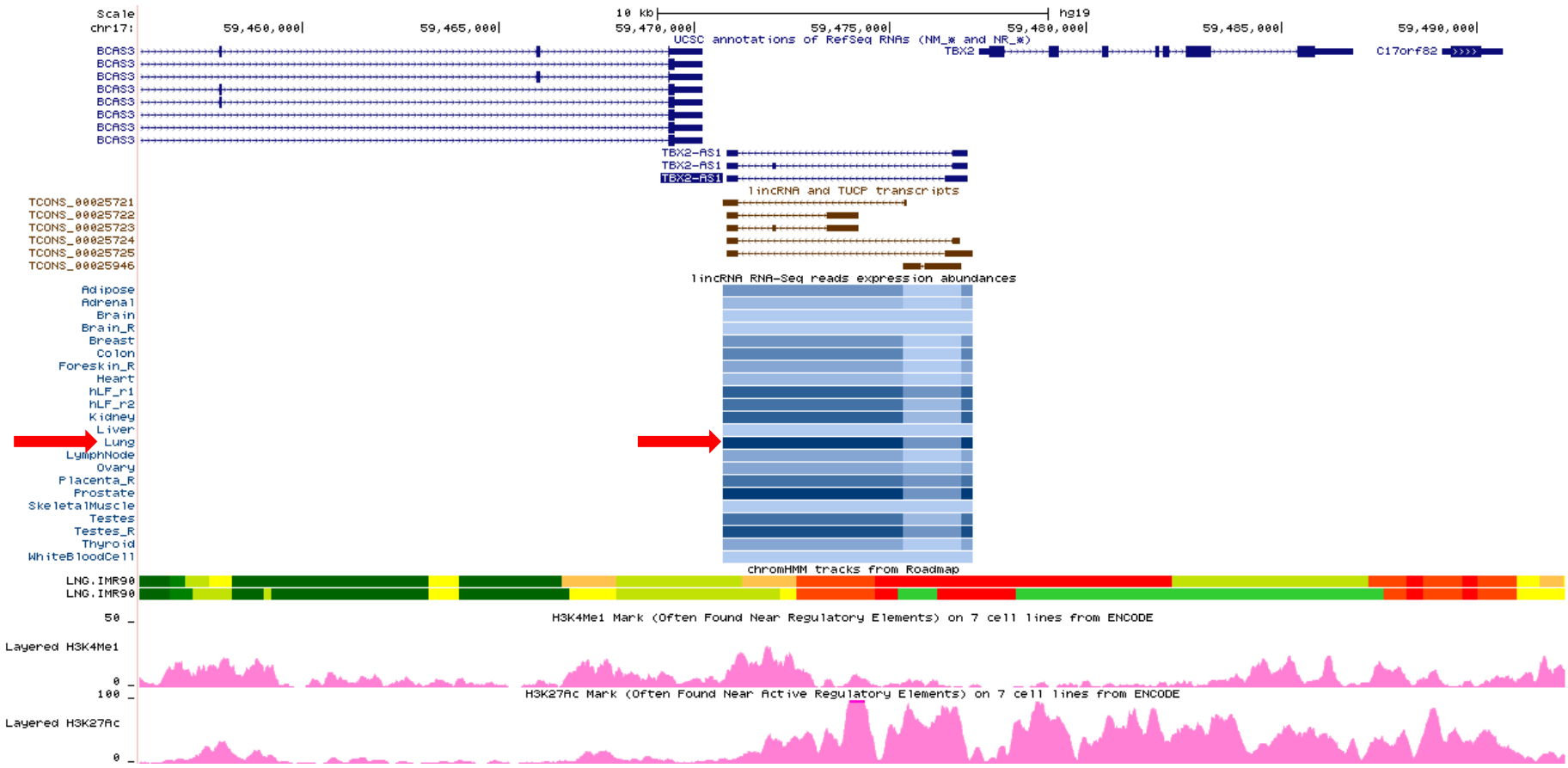


Figure S9. Schematic representation of the chromosomal region between the *BCAS3* and *TBX2* genes located within the deletion region. This graph shows non-coding transcripts and their expression in different types of tissues. Red arrows indicate the strong expression of lncRNA in the lung tissue. H3KMe1, and H3KMe3 marks in the human lung and chromatin state annotation based on ChIP-seq mapping (Roadmap) in the IMR-90 cell line are shown.

Table S1. Summary of cases of AcDys reported in the literature.

Author Year	Number of subjects (Gender)	Familial history	Prenatal findings	Survival	Anatomopathology	PAH	Extra-pulmonary features	Genetic findings
Rutledge 1986[3]	1 (F)	NA	Born at term	H7	Moderate lung hypoplasia (CLW=43g). Deranged air spaces lined by ciliated bronchial epithelium, no development of alveoli, increased amounts of intervening fibrous tissue.	NA	Right aortic arch, bilateral thinned renal cortices	NA
Chambers 1991[4]	1 (F)	1 healthy brother	US normal	hours	Lung hypoplasia (CLW=30g). Total failure of development of terminal respiratory units with arrest of pulmonary growth early in the second trimester.	NA	-	NA
Davidson 1998[5]	1 (F)	1 healthy brother, 1 healthy sister	US normal	hours	Lung hypoplasia (CLW=21.8g). Bronchial development but no acinar development, corresponding to the pseudoglandular phase of 16 weeks gestation.	NA	-	NA
Moerman 1998[2]	2 (F) (siblings)	1 healthy brother No consanguinity	Mild IUGR (10-25th centile)	D2/H24	Lung hypoplasia (CLW=20.2g/ 18.9g and LW/BW=0.007). Reduced alveolar parenchyma, complete absence of mature alveoli, increased amount of interstitial connective tissue, dysplastic bronchial cartilage plates. Stop early canalicular	-	-	Karyotype N
Al-Senan 2003[6]	1 (F)	2 sisters affected No consanguinity	Mild oligohydramnios	3 months	Only one of the three infants had an autopsy to confirm CPAM type 0, however the three females had similar presentations.	NA	NA	aCGH N
Gillespie 2004[7]	1 (M)	No siblings	Large renal mass, polyhydramnios, no IUGR	H4	Normal lung weight. Irregularly branching airspaces with a ciliated cuboidal lining. Capillaries in adjacent connective tissue were reduced in number. No normal alveoli. Abundant loose interstitial connective tissue. bilateral	No	Severe hydronephrosis, cystic renal dysplasia	NA
Stuhrmann 2007[8]	1 (F)	NA	NA	hours	CPAM type 0	NA	NA	NA
DeBoer 2012[9]	2 (F) (Twins)	1 affected brother (no autopsy) No consanguinity	NA	H9/D5	Twin A: LW/BW= 0.0098 (CLW=32g).Twin B: LW/BW=0.0095 (CLW=45g). Both: Pulmonary vasculature normal. Severe maturation arrest, terminal bronchioles, rare alveoli.	+, severe	Gallbladder agenesis, mild hydronephrosis bilaterally	NA
Langenstroer 2013[10]	1 (M)	NA	Mild IUGR, small chest circumference	D20	Lung hypoplasia, diffuse growth arrest without alveolar development.	+	Left frontal lobe cerebral infarct	Karyotype N
Chow 2013[11]	2 (M and F) (siblings)	3 healthy siblings. No consanguinity	US normal	H6/D24	Lung hypoplasia (Brother: LW/BW=0.007; sister: LW/BW=0.017). Spaces lined by ciliated columnar epithelium and separated by mesenchyme, with minimal saccule-like structures.	+, moderate	-	NA

Author Year	Number of subjects (Gender)	Familial history	Prenatal findings	Survival	Anatomopathology	PAH	Extra-pulmonary features	Genetic findings
Chow 2013[11]	1 (F)	1 sibling affected (no autopsy), Consanguinity (first cousins), 2 healthy siblings 2 siblings with MPSIII	US normal	Alive at 18 months	Incomplete form of acinar dysplasia.	+, persisting at 18 months	NA	Karyotype N; MPS type IIIA, *SPB* and *SPC* negative.
Don 2014[12]	1 (M)	1 healthy sister	US normal	H2	Congenital lung dysplasia. Developmental architecture was arrested in between the canalicular and saccular stages. All lung sections demonstrated tiny lobules with unexpanded to poorly expanded pulmonary parenchyma.	-	-	Karyotype N
Lertsburapa 2014[13]	1 (M)	NA	Intermittent bleeding between 13 and 20 weeks, bilateral fetal adrenal hemorrhage detected at 17W.	hours	Lung hypoplasia, exaggerated lobulation, focal acinar dysgenesis with arrest of development in the pseudoglandular stage. Focal lobular hyperplasia and microcystic maldevelopment.	+	small, calcified adrenal glands, remote cerebral and cerebellar infarcts.	Karyotype N
Szafranski 2016[1]	1 (F)	NA	US normal	D1	Lung hypoplasia (CLW=21g, LW/BW=0.007). Maldevelopment of the terminal bronchioles, respiratory bronchioles, and alveoli. Stop at pseudoglandular stage.	NA	-	De novo heterozygous variant c.256G>C, p.(E86Q) in exon 2 of *TBX4*
Barnett 2016[14]	1 (F)	Consanguinity (first cousins)	Limb malformations at 19W.	H5	Lung hypoplasia (CLW=26.89 g). Stop at pseudoglandular stage, multiple small cysts.	NA	Ectrodactyly, Dysmorphic facial features.	Homozygous variant c.764G>A, p.(R255Q) in *FGFR2*

Abbreviations are as follows: +, present; -, absent; aCGH, array comparative genomic hybridization; BW, body weight; CLW, combined lung weight; CPAM, congenital pulmonary airway malformation; D, day; F, female; H, hour; IUGR, intrauterine growth retardation; LW, lung weight; M, male; Min, minutes; N, normal; NA, not applicable; PAH, pulmonary arterial hypertension; US, ultrasounds.

Table S2. Clinical findings in the individuals involved in the study.

Separate file

Abbreviations are as follows: +, present; -, absent; AcDys, acinar dysplasia; BW, body weight; CAD, congenital alveolar dysplasia; CPAM - congenital pulmonary airway malformation; D, day; ECMO, extracorporal membrane oxygenation; F, female; G, gender; GA, gestational age; H, hour; IUGR, intrauterine growth restriction; LADD syndrome, lacrimoauriculodentodigital syndrome; LW, lung weight; M, male; Min, minutes; N, normal; NA - not available; PAH, pulmonary arterial hypertension.

Table S3. Genetic findings in studied individuals with lung hypoplasia

Subject	G	Ethnicity	Diagnosis	Deletion CNV coordinates (hg19)	Repetitive element at the breakpoints	Microhomology [bp]	SNV	Inheritance	WGS	ES	aCGH
a) 17q23.1q23.2 deletions involving entire *TBX4*											
P006	F	unk	AcDys	chr17:58,089,454/58,090,137-60,346,028/60,346,711	LCR/LCR	683	-	unk	x	x	x
P009	F	C	AcDys	chr17:58,090,283/58,090,656-60,346,857/60,347,230	LCR/LCR	373	-	de novo	x	x	x
P012	F	C	AcDys	chr17:58,088,933/58,089,453-60,345,508/60,346,028	LCR/LCR	520	-	de novo	x	x	x
P019	M	C	N/A	~chr17:58,167,485-60,174,066	LCR/LCR	unk	-	unk	x	-	x
P026	F	C	AcDys	chr17:58,088,933/58,089,453-60,345,508/60,346,028	LCR/LCR	520	*BCLAF1* (NM_001077440.1) c.1615G>A, p.(Asp539Asn)	unk	x	x	x
P073	F	C	N/A	chr17:58,086,876/58,087,936-60,343,456/60,344,516	LCR/LCR	1060	-	de novo	x	-	x
P035	M	C	AcDys	chr17:59,272,842/59,272,846-61,392,993/61,392,997	*Alu*Jb/-	4	-	unk	x	-	x
b) *TBX4* intragenic deletion at 17q23.2											
P015/P016	M/F	C	CAD/AcDys spectrum	chr17:59,542,891/59,542,894-59,551,500/59,551,503	-/-	3	*TBX5* (NM_000192.3) c.331G>T, p.(Asp111Tyr)	inherited from the mother (CNV) and the father (SNV)	x	x	x
c) *TBX4* point mutations											
P022	M	C	AcDys	N/A[I]	N/A	N/A	*TBX4* (NM_018488.3) c.256G>A, p.(Glu86Lys)	de novo	x	-	-
P025	F	unk	Marked variation with AcDys ranging to near normal	N/A	N/A	N/A	*TBX4* (NM_018488.3) c.256G>C, p.(Glu86Gln)	de novo	x	x	-
d) 17q23 deletions involving *BCAS3*											
P038	M	unk	AcDys	chr17:58,857,889/58,857,898-58,868,328/58,868,337	*Alu*Sx1/*Alu*Sx	9	-	inherited from the father	x	-	x
e) 5p12 deletions involving *FGF10*											
P040/P041	F/F	C	Pulmonary hypoplasia/ CAD vs	chr5:43,957,152/43,957,220-46,135,141/46,135,209	L1PA4/L1PA4	68	-	inherited from the mother(P04	x	-	-

			pulmonary hypoplasia					0)/ father(P041)			
P076	F	unk	Pulmonary hypoplasia	chr5:42,985,023-45,244,787	-/L1PA15	0	-	inherited from the father	x	-	-
f) *FGF10* mutations											
P033	F	C	AcDys	N/A	N/A	N/A	*FGF10* (NM_004465.1) c.526delA, p.(Met176Cysfs*5) *STRA6* (NM_001142617.1) c.653T>C p.(Phe218Ser)	inherited from the father inherited from the mother	x	x	x
P042	F	C	CAD	N/A	N/A	N/A	*FGF10* (NM_004465.1) c.577C>T, p.(Arg193*) *FRAS1* (NM_025074.6) c.10245G>C p.(Gln3415His)	unk	x	x	x
g) other mutations											
P003	F	C	Marked variation with AcDys ranging to near normal	N/A	N/A	N/A	*BTBD7* (NM_018167.4) c.1075G>A, p.(Ala359Thr) *FRAS1* (NM_025074.6) c.4648C>T, p.(Leu1550Phe) c.7039G>T, p.(Val2347Phe)	unk	x	x	x
P027	M	C	AcDys	N/A	N/A	N/A	*FRAS1* (NM_025074.6) c.7451C>T, p.(Thr2484Met)	unk	x	x	-
P028	F	C	AcDys	N/A	N/A	N/A	*DSPP* (NM_014208.3) c.3660_3661insATCT, p. Asp1221Ilefs*2) c.3734_3742delGACAGCAG, p.(Asn1248_Ser1250del)	unk	x	x	-
P046	M	unk	AcDys	N/A	N/A	N/A	*TCF21* (NM_003206.3) c.329C>T, p.(Pro110Leu)	de novo	x	x	x
h) no genetic findings											
P034	F	C	CAD versus pulmonary hypoplasia	-	N/A	N/A	-	N/A	x	-	-
P043	M	C	AcDys	-	N/A	N/A	-	N/A	x	x	x
P044	F	C	n/a	-	N/A	N/A	-	N/A	x	x	x
P045	F	C	AcDys	-	N/A	N/A	-	N/A	x	x	x
P048	F	N-A^{m}	Pulmonary hypoplasia	-	N/A	N/A	-	N/A	x	x	x

Abbreviations are as follows: aCGH, array comparative genomic hybridization; AcDys, acinar dysplasia; C, Caucasian; CAD, congenital alveolar dysplasia; CNV, copy number variant; ES, exome sequencing; G, gender; LCR, low-copy repeats; N-A, North African; NA, not applicable; SNV, single nucleotide variant; unk, unknown; WGS, whole genome sequencing.

Table S4. ES findings in studied affected individuals.

Subject	Gene (NM number)	Variant	rs[a]	Mutation Taster	Poly Phen2	gnomAD[b] MAF
P003	*BTBD7* (NM_018167.4)	c.G1075A p.(Ala359Thr)	rs61747488	D	P	0.00001220
	FRAS1 (NM_025074.6)	c.C4648T p.(Leu1550Phe)	rs148663672	D	D	0.002372
		c.G7039T p.(Val2347Phe)	rs201369510	D	B	0.001395
P015	*TBX5* (NM_000192.3)	c.331G>T p.(Asp111Tyr)	rs77357563	D	D	0.003304
P016	*TBX5* (NM_000192.3)	c.331G>T p.(Asp111Tyr)	rs77357563	D	D	0.003304
P025	*TBX4* (NM_018488.3)	c.256G>C p.(Glu86Gln)	NA	D	P	NA
P026	*BCLAF1* (NM_001077440.1)	c.1615G>A p.(Asp539Asn)	rs201061168	D	D	0.000004061
P027	*FRAS1* (NM_025074.6)	c.7451C>T p.(Thr2484Met)	rs200888184	D	P	0.0004448
P028	*DSPP* (NM_014208.3)	c.3660_3661insATCT p.(Asp1221Ilefs*2)	NA	NA	NA	NA
		c.3734_3742delGACAGCAG p.(Asn1248_Ser1250del)	NA	NA	NA	NA
P033	*FGF10* (NM_004465.1)	c.526delA p.(Met176Cysfs*5)	NA	NA	NA	NA
	STRA6 (NM_001142617.1)	c.653T>C p.(Phe218Ser)	rs764331156	P	P	0.00003535
P042	*FGF10* (NM_004465.1)	c.577C>T p.(Arg193*)	rs104893884	NA	NA	NA
	FRAS1 (NM_025074.6)	c.10245G>C p.(Gln3415His)	rs746969511	D	D	0.000008140
P046	*TCF21* (NM_003206.3)	c.329C>T p.(Pro110Leu)	NA	D	D	NA

Abbreviations are as follows: Alt, altered allele; B, benign; D, damaging; MAF, minor allele frequency; n/a, not applicable; P, possibly damaging; Ref, reference allele. [a]rs numbers based on dbSNP v.150; [b]MAF based the GnomAD database (r2.0.2).

Table S5. Calculated total AOH sizes in studied affected individuals.

Subject	AOH size (bp)	Consanguinity
P003	18,206,669	-
P009	10,033,523	-
P012	19,216,060	-
P015	10,664,287	-
P025	7,484,562	-
P026	24,985,079	-
P027	6,517,738	-
P028	26,235,634	-
P033	NA	NA
P042	12,816,726	-
P043	34,236,336	-
P044	9,489,842	-
P045	17,062,337	-
P046	9,767,608	-
P048	349,621,354	+

Abbreviations are as follows: +, present; -, absent; AOH, absence of heterozygosity; NA, not applicable.

Table S6. Results of SNP array.

Separate file

Abbreviations are as follows: Chr, chromosome; SNP, single nucleotide polymorphism; [a]rs numbers based on dbSNP v.150.

Table S7. Non-coding variants identified within *TBX4* (NM_018488.3) in affected individuals with truncating *TBX4* mutations.

Genomic coordinates (hg19) Chr17			**59,544,058**	**59,544,863**	**59,545,329**	**59,545,750**	**59,545,838**	**59,546,366**
Ref/Alt			**A/T**	**G/A**	**C/A**	**G/A**	**G/T**	**G/T**
rs#[a]			**rs6504044**	**rs758596**	**rs873363**	**rs7214481**	**rs7214641**	**rs8076015**
Subjects	17q23 deletion	P035	wt	wt	wt	wt	wt	wt
		P019	wt	wt	hem	wt	wt	wt
		P026	hem	hem	hem	hem	hem	hem
		P006	wt	wt	wt	wt	wt	wt
		P009	hem	wt	wt	wt	wt	wt
		P012	wt	wt	wt	wt	wt	wt
		P073	hem	hem	hem	hem	hem	hem
	Intragenic *TBX4* deletion	P015	hem	hem	hem	hem	hem	hem
		P016	hem	hem	hem	hem	hem	hem
	TBX4 SNV	P025	wt	wt	het	wt	wt	wt
		P022	het	het	het	het	het	het
Controls	17q23 deletion	C059	wt	wt	wt	wt	wt	wt
		C058	wt	wt	wt	wt	wt	wt
		C051	wt	wt	wt	wt	wt	wt
		C055	wt	wt	wt	wt	wt	wt
		C054	wt	wt	wt	wt	wt	wt
		C052	wt	wt	wt	wt	wt	wt
		C060	hem	hem	hem	hem	hem	hem
		C061	hem	hem	hem	hem	hem	hem
		C062	wt	wt	wt	wt	wt	wt
		C063	wt	wt	wt	wt	wt	wt
		C064	wt	wt	wt	wt	wt	wt
		C065	wt	wt	wt	wt	wt	wt
		C072	wt	wt	wt	wt	wt	wt
	Intragenic *TBX4* deletion	C079	wt	wt	wt	wt	wt	wt

Abbreviations are as follows: Alt, altered allele; hem, hemizygous; het, heterozygous; Ref, reference allele; SNV, single nucleotide variant; wt, wild type. [a]rs numbers based on dbSNP v.150.

Table S8. Block of 21 SNPs haplotype common in affected subjects P040 and P041.

Chr	Start	End	Ref	Alt	rs#[a]	Ref Gene	C039	P040	P041	C074	P076	C077
chr5	44567410	44567410	G	A	rs13182481	intergenic	wt	hem	hem	hem	hem	wt
chr5	44568655	44568655	A	G	rs4463187	intergenic	wt	hem	hem	hem	hem	wt
chr5	44576171	44576171	A	C	rs10054521	intergenic	wt	hem	hem	hem	hem	wt
chr5	44578164	44578164	A	C	rs9765572	intergenic	wt	hem	hem	hem	hem	wt
chr5	44578165	44578165	C	T	rs9764095	intergenic	wt	hem	hem	hem	hem	wt
chr5	44580193	44580193	C	A	rs4866909	intergenic	wt	hem	hem	hem	hem	wt
chr5	44581194	44581194	A	C	rs10053984	intergenic	wt	hem	hem	hem	hem	wt
chr5	44587238	44587238	T	C	rs10059745	intergenic	wt	hem	hem	hem	hem	wt
chr5	44590910	44590910	G	A	rs6862655	intergenic	wt	hem	hem	hem	hem	wt
chr5	44591815	44591815	C	T	rs4348227	intergenic	wt	hem	hem	hem	hem	wt
chr5	44591995	44591995	G	A	rs4639238	intergenic	wt	hem	hem	hem	hem	wt
chr5	44594460	44594461	AC	-	rs35053942	intergenic	wt	hem	hem	hem	hem	wt
chr5	44600996	44600996	T	G	rs10066953	intergenic	wt	hem	hem	hem	hem	wt
chr5	44604313	44604313	G	A	rs12374507	intergenic	wt	hem	hem	hem	hem	wt
chr5	44606379	44606379	A	G	rs6892239	intergenic	wt	hem	hem	hem	hem	wt
chr5	44609841	44609841	G	A	rs10065325	intergenic	wt	hem	hem	hem	hem	wt
chr5	44611650	44611650	A	G	rs4573006	intergenic	wt	hem	hem	hem	hem	wt
chr5	44620819	44620819	A	G	rs9654396	intergenic	wt	hem	hem	hem	hem	wt
chr5	44626810	44626810	G	T	rs6866354	intergenic	wt	hem	hem	hem	hem	wt
chr5	44853593	44853593	G	C	rs17343002	intergenic	wt	hem	hem	hem	wt	wt
chr5	45333860	45333860	T	C	rs55821517	*HCN1*, intronic	wt	hem	hem	het	het	wt

Abbreviations are as follows: Alt, altered allele; hem, hemizygous; het, heterozygous; Ref, reference allele; SNV, single nucleotide variant; wt, wild type. [a]rs numbers based on dbSNP v.150.

Table S9. Analysis of eQTL.

Separate file

Abbreviations are as follows: Alt, altered allele; Chr, chromosome; hem, hemizygous; het, heterozygous; Ref, reference allele; wt, wild type. [a]rs numbers based on dbSNP v.150.

Table S10. Overview of the published deletions in the 17q23.1q23.2 region.

Phenotype	Subject[a]	Position hg19 [Mb]
Heart Defects, Limb Abnormalities, DD, and PAH	Pt 3, 4, 7[15]	~58.0-60.2
Heart Defects, Limb Abnormalities, DD	Pt 1, 5, 6[15]	~58.0-60.2
Heart Defects, Limb Abnormalities, DD	Pt 2[15]	~57.4-60.2
DD, PAH and sensorineural hearing loss	Pt 1[16]	~56.4-60.2
DD, PAH and sensorineural hearing loss	Pt 1[17]	~58.1-60.2
DD, PAH and sensorineural hearing loss	Pt 2[17]	~58.1-60.3
SPS and PAH	Pt 1[18]	~58.0-60.2
SPS and PAH	Pt 2[18]	~59.2.-61.2
SPS and PAH	Pt 3[18]	~58.1-60.2
PAH	Pt[19]	~59.53-59.56

Abbreviations are as follows: DD, developmental delay; PAH, pulmonary hypertension; SPS, small patella syndrome. [a]Number of subject in the original publication.

References:

1. Szafranski, P., Coban-Akdemir, Z.H., Rupps, R., Grazioli, S., Wensley, D., Jhangiani, S.N., Popek, E., Lee, A.F., Lupski, J.R., Boerkoel, C.F., et al. (2016). Phenotypic expansion of TBX4 mutations to include acinar dysplasia of the lungs. Am. J. Med. Genet. A *170*, 2440–2444.

2. Moerman, P., Vanhole, C., Devlieger, H., and Fryns, J.P. (1998). Severe primary pulmonary hypoplasia ("acinar dysplasia") in sibs: a genetically determined mesodermal defect? J. Med. Genet. *35*, 964–965.

3. Rutledge, J.C., and Jensen, P. (1986). Acinar dysplasia: a new form of pulmonary maldevelopment. Hum. Pathol. *17*, 1290–1293.

4. Chambers, H.M. (1991). Congenital acinar aplasia: an extreme form of pulmonary maldevelopment. Pathology *23*, 69–71.

5. Davidson, L.A., Batman, P., and Fagan, D.G. (1998). Congenital acinar dysplasia: a rare cause of pulmonary hypoplasia. Histopathology *32*, 57–59.

6. Al-Senan, K.A., Kattan, A.K., and Al-Dayel, F.H. (2003). Congenital acinar dysplasia. Familial cause of a fatal respiratory failure in a neonate. Saudi Med J *24*, 88–90.

7. Gillespie, L.M., Fenton, A.C., and Wright, C. (2004). Acinar dysplasia: a rare cause of neonatal respiratory failure. Acta Paediatr. *93*, 712–713.

8. Stuhrmann, S., Sachweh, J., Bindl, L., Vázquez-Jiménez, J., Hermanns-Sachweh, B., and Seghaye, M.-C. (2007). Congenital cystic adenomatoid malformation type 0-a rare cause of neonatal death. Pediatr Crit Care Med *8*, 580–581.

9. DeBoer, E.M., Keene, S., Winkler, A.M., and Shehata, B.M. (2012). Identical twins with lethal congenital pulmonary airway malformation type 0 (acinar dysplasia): further evidence of familial tendency. Fetal Pediatr Pathol *31*, 217–224.

10. Langenstroer, M., Carlan, S.J., Fanaian, N., and Attia, S. (2013). Congenital acinar dysplasia: report of a case and review of literature. AJP Rep *3*, 9–12.

11. Chow, C.W., Massie, J., Ng, J., Mills, J., and Baker, M. (2013). Acinar dysplasia of the lungs: variation in the extent of involvement and clinical features. Pathology *45*, 38–43.

12. Don, M., Orsaria, M., Da Dalt, E., Tringali, C., and Sacher, B. (2014). Rapidly fatal "congenital lung dysplasia": a case report and review of the literature. Fetal Pediatr Pathol *33*, 109–113.

13. Lertsburapa, T., Vargas, D., Lambert-Messerlian, G., Tantravahi, U., Gündoğan, F., DeLaMonte, S., Coyle, M.G., and De Paepe, M.E. (2014). Lethal hypoplasia and developmental anomalies of the lungs in a newborn with intrauterine adrenal hemorrhage and cerebral infarcts: a proposed pulmonary disruption sequence. Pediatr. Dev. Pathol. *17*, 374–381.

14. Barnett, C.P., Nataren, N.J., Klingler-Hoffmann, M., Schwarz, Q., Chong, C.-E., Lee, Y.K., Bruno, D.L., Lipsett, J., McPhee, A.J., Schreiber, A.W., et al. (2016). Ectrodactyly and Lethal Pulmonary Acinar Dysplasia Associated with Homozygous FGFR2 Mutations Identified by Exome Sequencing. Hum. Mutat. *37*, 955–963.

15. Ballif, B.C., Theisen, A., Rosenfeld, J.A., Traylor, R.N., Gastier-Foster, J., Thrush, D.L., Astbury, C., Bartholomew, D., McBride, K.L., Pyatt, R.E., et al. (2010). Identification of a recurrent microdeletion at 17q23.1q23.2 flanked by segmental duplications associated with heart defects and limb abnormalities. Am. J. Hum. Genet. *86*, 454–461.

16. Nimmakayalu, M., Major, H., Sheffield, V., Solomon, D.H., Smith, R.J., Patil, S.R., and Shchelochkov, O.A. (2011). Microdeletion of 17q22q23.2 encompassing TBX2 and TBX4 in a patient with congenital microcephaly, thyroid duct cyst, sensorineural hearing loss, and pulmonary hypertension. Am. J. Med. Genet. A *155A*, 418–423.

17. Schönewolf-Greulich, B., Ronan, A., Ravn, K., Baekgaard, P., Lodahl, M., Nielsen, K., Rendtorff, N.D., Tranebjaerg, L., Brøndum-Nielsen, K., and Tümer, Z. (2011). Two new cases with microdeletion of 17q23.2 suggest presence of a candidate gene for sensorineural hearing loss within this region. Am. J. Med. Genet. A *155A*, 2964–2969.

18. Kerstjens-Frederikse, W.S., Bongers, E.M.H.F., Roofthooft, M.T.R., Leter, E.M., Douwes, J.M., Van Dijk, A., Vonk-Noordegraaf, A., Dijk-Bos, K.K., Hoefsloot, L.H., Hoendermis, E.S., et al. (2013). TBX4 mutations (small patella syndrome) are associated with childhood-onset pulmonary arterial hypertension. J. Med. Genet. *50*, 500–506.

19. Zhu, N., Gonzaga-Jauregui, C., Welch, C.L., Ma, L., Qi, H., King, A.K., Krishnan, U., Rosenzweig, E.B., Ivy, D.D., Austin, E.D., et al. (2018). Exome Sequencing in Children With Pulmonary Arterial Hypertension Demonstrates Differences Compared With Adults. Circ Genom Precis Med *11*, e001887.