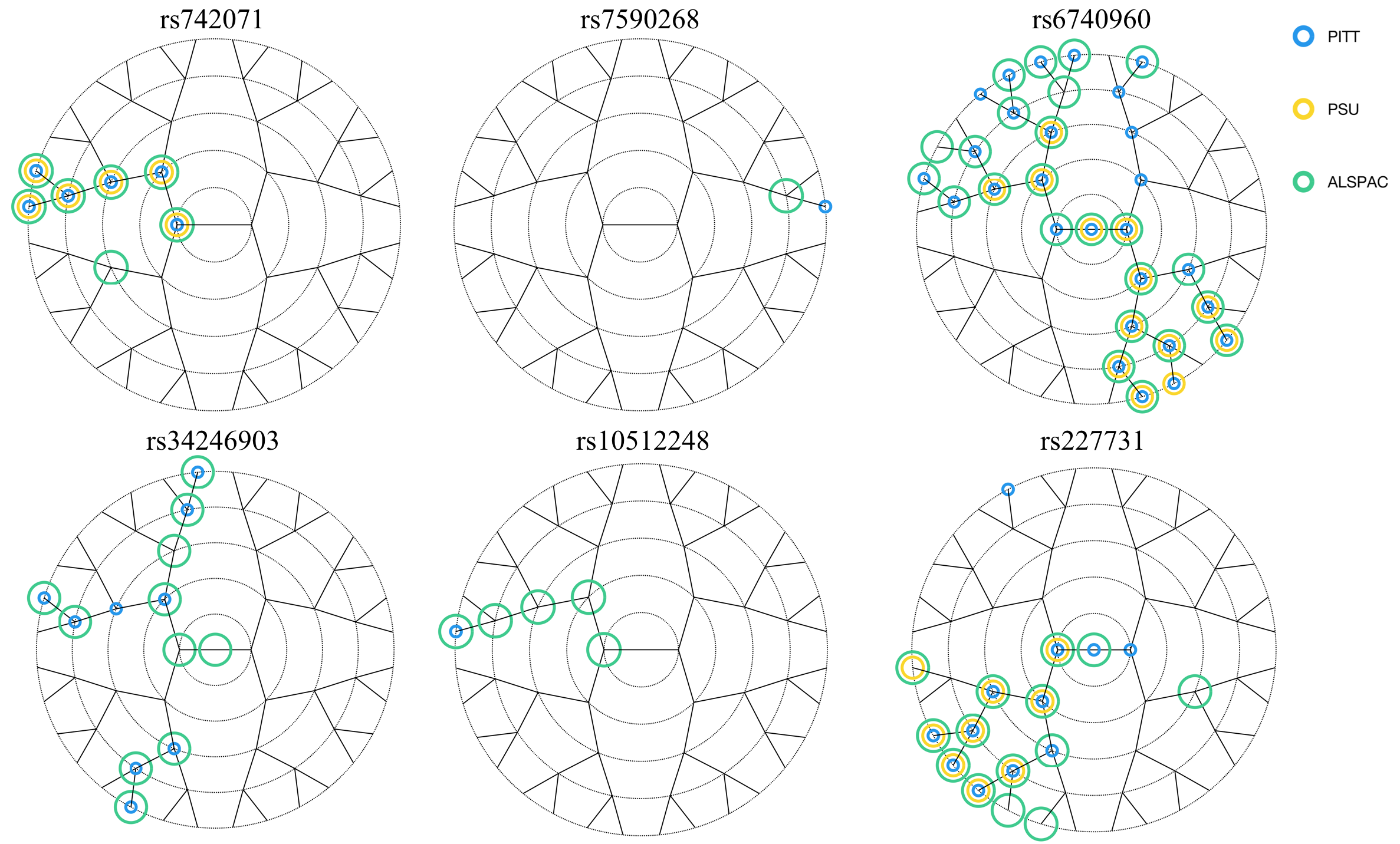
Supplementary Material

Four NSCL/P loci associated with normal craniofacial morphology.

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# Supplementary Figures



**Supplementary Figure 1: Meta-analysis results. The segments are encircled when significant (p-value < 8.04x10-6) in the meta-analysis with the color-coded dataset as discovery dataset.**

1. **Supplementary Text**

## Significant variants in literature

rs742071 in 1p36 is an intron variant of *PAX7*, which encodes a paired box domain transcription factor that plays a role in neural crest development (Basch, Bronner-Fraser, and García-Castro 2006). Neural crest cells are an ectoderm-derived multipotent cell population that gives rise to various cell lineages, including craniofacial cartilage. Mutant mice with impaired Pax7 function display severe craniofacial defects, showing malformations in the facial structures involving the maxilla and nose (Mansouri et al. 1996; Zalc et al. 2015).

rs7590268 is an intron variant of *THADA*, a gene associated with polycystic ovary syndrome and the target of 2p21 chromosomal aberrations in benign thyroid adenomas (Goodarzi et al. 2012; Day et al. 2015; Rippe et al. 2003). In contrast to all other loci mentioned above, this SNP has no previous findings in craniofacial development, except for the association with NSCL/P (Ludwig et al. 2012).

The strongest effect was found for rs6740960 on 2p21, which lies in a non-coding region. This effect was also found by Claes *et al.* (2018), where the SNP was found to be located within an active craniofacial neural crest cell enhancer. The SNP maps 100 kb upstream of *PKDCC*, a possible candidate gene that encodes the cytoplasmic protein kinase domain containing protein. In zebrafish *PKDCC*-orthologs have been shown to be involved in craniofacial development (Melvin et al. 2013). Although both rs7590268 and rs6740960 are localized in 2p21, the effects found in this study were independent. When identifying these SNPs in a NSCL/P case-control GWAS, Ludwig *et al.* (2017) used conditional analysis to show the effects were independent from rs6740960. Additionally, the effects of the two SNPs were located in two different facial areas.

rs34246903 is located 50 kb upstream of *MSX1*, which encodes a transcription factor upregulated during embryogenesis and postnatal development in bone tissue. *BMP* expression in palatal mesenchyme is regulated by the *MSX1* homeobox gene and plays an import role in epithelial-mesenchymal interaction throughout embryogenesis (Satokata and Maas 1994).

rs10512248 is an intron variant of *Patched1* or *PTCH1*. This gene encodes a receptor in the Hedgehog signaling pathway and plays a major role in craniofacial development (Aoto and Trainor 2015).

In 17q22, rs227731 *NOG*, which encodes a BMP antagonist. NOG has been found to play a role in neural crest formation by regulating BMP signaling (Anderson et al. 2006). Matsui & Klingensmith (2014) found evidence in mouse studies that NOGis required in neural crest cells for palatal development, and that *NOG* is expressed during early craniofacial development and acts in neural crest cells to regulate mandible shape and size.

## Supplementary Tables

**Supplementary Table 1: Discovery and meta-analysis results of all SNPs in all segments. Minor allele < major allele. CC, canonical correlation. The CCA-correlations are listed in the order of Pittsburgh, Penn State and ALSPAC, the same order is given for the discovery p-values and the meta-analysis p-values. Supplementary Table in separate Excel File**

**Supplementary Table 2: Linkage Disequilibrium in 1p36 calculated with the NCI NIH analysis tool LDmatrix in LDlink (Machiela and Chanock 2015).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | rs9439713 | | rs4920524 | | rs742071 | |
|  | D' | R2 | D' | R2 | D' | R2 |
| rs9439713 | 1.0 | 1.0 | 0.992 | 0.984 | 0.991 | 0.873 |
| rs4920524 | 0.992 | 0.984 | 1.0 | 1.0 | 1.0 | 0.888 |
| rs742071 | 0.991 | 0.873 | 1.0 | 0.888 | 1.0 | 1.0 |

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