

Inherited deletions!

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Abstract

Copy number variants (CNVs) may play an important part in the development of common birth defects such as oral clefts, and individual patients with multiple birth defects (including clefts) have been shown to carry small and large chromosomal deletions. In this paper we investigate the transmission rates of deletions in cleft offspring-parent trios.

Background (from Manuscript #1)

Oral clefts are among the most common birth defects, and include three anatomical defects: cleft lip (CL); cleft lip and palate (CLP) and cleft palate (CP). Because there are similarities in embryological and epidemiological evidence, CL and CLP are often grouped together as cleft lip with/without cleft palate (CL/P), although there is debate about whether all three groups may have distinct etiologies [1, 2]. Collectively, oral clefts represent half of all craniofacial malformations and create a major public health burden for both affected children and their families. The overall prevalence of oral clefts is estimated at 1 per 700 live births worldwide, but there is dramatic variation across populations and between racial and ethnic groups, in particular for CL/P [3, 4].

Oral clefts show strong familial aggregation, and the recurrence risk among first degree relatives is approximately 32 times greater than the general population risk for CL/P, and approximately 56 times greater for CP [5]. Twin studies also suggest a major role for genes controlling risk to oral clefts with monozygotic twins showing much higher concordance rates than dizygotic twins, 31% versus 2% for CL/P, and 43% versus 7% for CP [6]. Normal development of craniofacial features is a complex process and disruption of any one of numerous steps can lead to development of oral clefts [7], further supported by mounting evidence that multiple genes, in addition to environmental influences, play a role in the etiology of oral clefts [8, 9, 10, 11, 12, 13].

Assessment of chromosomal anomalies such as microdeletions and translocations also played an important role in the identification of genes and genomic regions underlying facial disorders [14, 15, 16, 17, 18, 19, 20, 21, 22]. In particular, high throughput technologies such as CGH and SNP arrays have gained popularity in assessing chromosomal alterations [23, 24]. For example, Sivertsen et.al assessed the prevalence of duplications and deletions in the 22q11 region (DiGeorge syndrome region) in Norwegian offspring with open CP, but did not detect an association [25]. Shi et. al used SNP genotyping, DNA sequencing, high-resolution DNA microarray analysis, and long-range PCR to characterize deletions in 333 candidate genes for orofacial clefting using 2,823 samples from 725 two and three-generation families ascertained through a proband having CL/P [26]. The authors confirmed several de-novo deletions in some of these candidate genes, in particular SUMO1, TBX1, and TFAP2A, raising the possibility that genes or regulatory elements contained within deleted regions might play a role in the etiology of oral clefts. Further, high rates of Mendelian inconsistencies were observed in 11 different genes, suggesting the existence of additional micro-deletions.

Results and Discussion

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Conclusions

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Methods

Data Description

- Performed 1346 tests. Bonferroni significant locus has width 140.733 kB.
- *PennCNV* joint HMM
- european, MAD < 0.3, non-WGA, aux \neq 1
- coverage > 10
- 13140 hemi/homozygous deletions identified in 445 trios
- 4288 CNV components

Common (> 0.01): 954

Rare: 3334

- Construct trio-states for all CNV components

recall that we use indicator variable for hemi/homozygous deletions

- must be at least 5 informative mating pairs

01x and 10x

- count transmissions and perform `binom.test` (See “trans.tab”)

In Figure 1 the $-\log_{10} p$ -values are displayed for the region on chromosome 8 that showed genome-wide significance after Bonferroni correction. We see that the frequency of the CNV components responsible for this signal have a frequency in the parents of nearly 0.5.

```
> table.list[which(trans.vec <= 0.05/n.tests)][[1]]
```

```
000 001 010 011 100 101 110 111
```

```
149   7  26  57  28  71   5 102
```

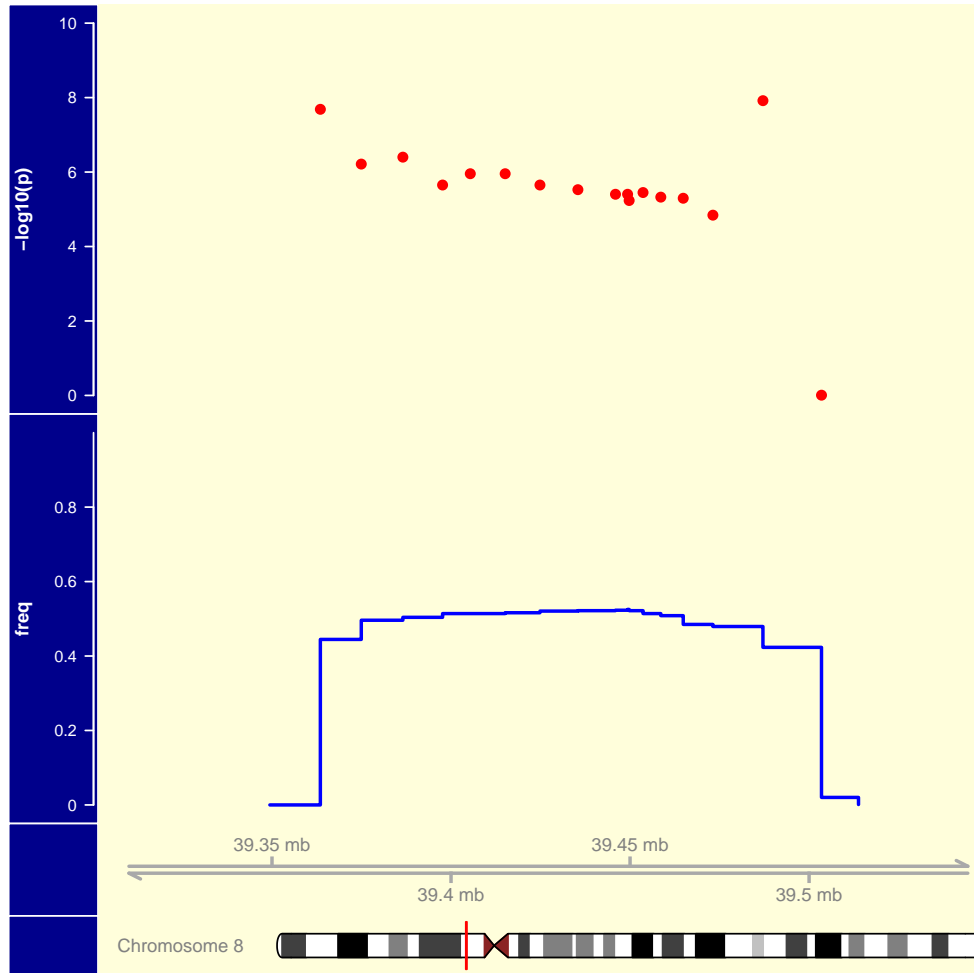


Figure 1: $-\log p$ -values for Fisher's exact test (blue) for an increase in transmission rate among cleft and control groups. The transmission rate in the cleft group (red) is seen to increase around 20 MB with the $\log p$ -value climbing to close to 3.

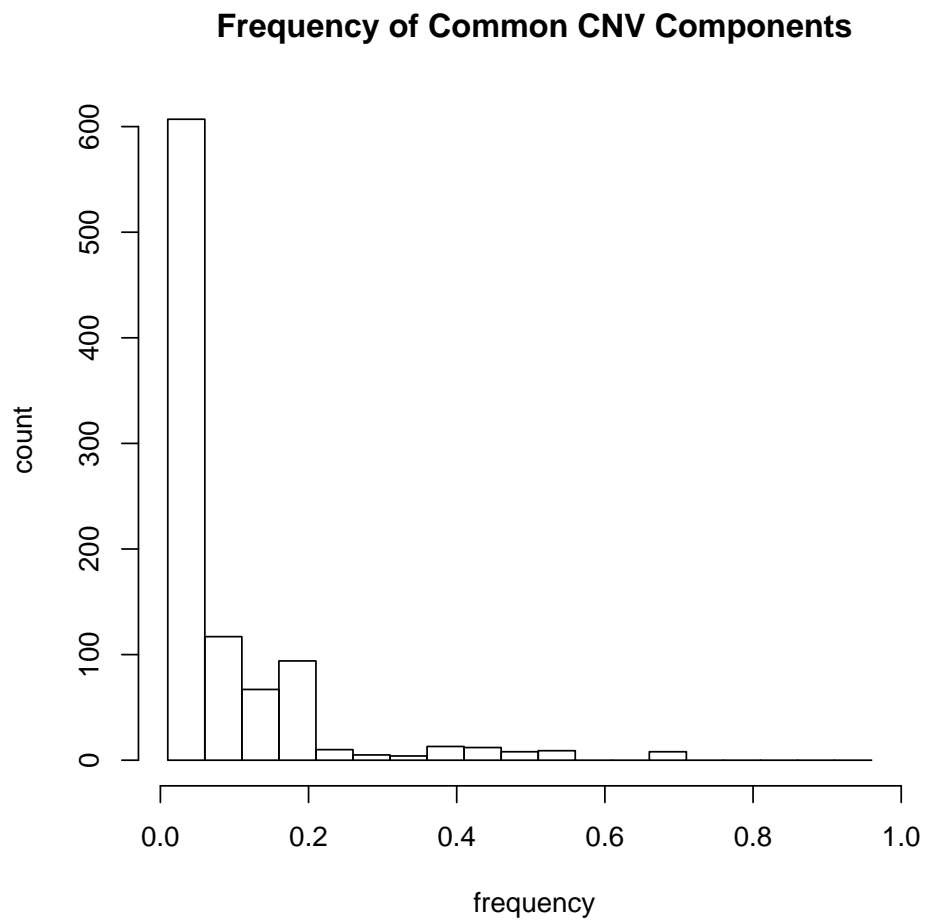


Figure 2: histogram

Authors contributions

Acknowledgements

References

1. Harville EW, Wilcox AJ, Lie RT, Vindenes H, Abyholm F: **Cleft lip and palate versus cleft lip only: are they distinct defects?** *Am J Epidemiol* 2005, **162**(5):448–453.
2. Forrester MB, Merz RD: **Comparison of cleft lip only and cleft lip and palate, Hawai'i, 1986–2003.** *Hawaii Med J* 2007, **66**(11):298, 300–298, 302.
3. Mossey P, Little J: *Cleft lip palate: from origin to treatment*, Oxford University Press 2002 chap. Epidemiology of oral clefts: an international perspective, :127–158.
4. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC: **Cleft lip and palate.** *Lancet* 2009, **374**(9703):1773–1785.
5. Sivertsen A, Wilcox AJ, Skjaerven R, Vindenes HA, Abyholm F, Harville E, Lie RT: **Familial risk of oral clefts by morphological type and severity: population based cohort study of first degree relatives.** *BMJ* 2008, **336**(7641):432–434.
6. Mitchell LE: *Cleft lip palate: from origin to treatment*, Oxford University Press 2002 chap. Twin studies in oral cleft research, :214–221.
7. Stanier P, Moore GE: **Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts.** *Hum Mol Genet* 2004, **13 Spec No 1**:R73–R81.
8. Farrall M, Holder S: **Familial recurrence-pattern analysis of cleft lip with or without cleft palate.** *Am J Hum Genet* 1992, **50**(2):270–277.
9. Schliekelman P, Slatkin M: **Multiplex relative risk and estimation of the number of loci underlying an inherited disease.** *Am J Hum Genet* 2002, **71**(6):1369–1385.
10. Jugessur A, Farlie PG, Kilpatrick N: **The genetics of isolated orofacial clefts: from genotypes to subphenotypes.** *Oral Dis* 2009, **15**(7):437–453.
11. Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, Liang KY, Wu T, Murray T, Fallin MD, Redett RA, Raymond G, Schwender H, Jin SC, Cooper ME, Dunnwald M, Mansilla MA, Leslie E, Bullard S, Lidral AC, Moreno LM, Menezes R, Vieira AR, Petrin A, Wilcox AJ, Lie RT, Jabs EW, Wu-Chou YH, Chen PK, Wang H, Ye X, Huang S, Yeow V, Chong SS, Jee SH, Shi B, Christensen K, Melbye M, Doheny KF, Pugh EW, Ling H, Castilla EE, Czeizel AE, Ma L, Field LL, Brody L, Pangilinan F, Mills JL, Molloy AM, Kirke PN, Scott JM, Scott JM, Arcos-Burgos M, Scott AF: **A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4.** *Nat Genet* 2010, **42**(6):525–529.
12. Dixon MJ, Marazita ML, Beaty TH, Murray JC: **Cleft lip and palate: understanding genetic and environmental influences.** *Nature reviews Genetics* 2011, **12**(3):167–178.
13. Ludwig KU, Mangold E, Herms S, Nowak S, Reutter H, Paul A, Becker J, Herberz R, AlChawa T, Nasser E, B  hmer AC, Mattheisen M, Alblas MA, Barth S, Kluck N, Lauster C, Braumann B, Reich RH, Hemprich A, P  tzsch S, Blaumeiser B, Daratsianos N, Kreusch T, Murray JC, Marazita ML, Ruczinski I, Scott AF, Beaty TH, Kramer FJ, Wienker TF, Steegers-Theunissen RP, Rubini M, Mossey PA, Hoffmann P, Lange C, Cichon S, Propping P, Knapp M, N  then MM: **Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci.** *Nat Genet* 2012, **44**(9):968–971.
14. Bocian M, Walker AP: **Lip pits and deletion 1q32–41.** *Am J Med Genet* 1987, **26**(2):437–443.
15. Sander A, Schmelzle R, Murray J: **Evidence for a microdeletion in 1q32–41 involving the gene responsible for Van der Woude syndrome.** *Hum Mol Genet* 1994, **3**(4):575–578.
16. Sander A, Murray JC, Scherpbier-Heddema T, Buetow KH, Weissenbach J, Zingg M, Ludwig K, Schmelzle R: **Microsatellite-based fine mapping of the Van der Woude syndrome locus to an interval of 4.1 cM between D1S245 and D1S414.** *Am J Hum Genet* 1995, **56**:310–318.
17. Brewer C, Holloway S, Zawalnyski P, Schinzel A, FitzPatrick D: **A chromosomal deletion map of human malformations.** *Am J Hum Genet* 1998, **63**(4):1153–1159.
18. Brewer C, Holloway S, Zawalnyski P, Schinzel A, FitzPatrick D: **A chromosomal duplication map of malformations: regions of suspected haplo- and triplolethality—and tolerance of segmental aneuploidy—in humans.** *Am J Hum Genet* 1999, **64**(6):1702–1708.

19. Schutte BC, Murray JC: **The many faces and factors of orofacial clefts.** *Hum Mol Genet* 1999, **8**(10):1853–1859.
20. FitzPatrick DR, Carr IM, McLaren L, Leek JP, Wightman P, Williamson K, Gautier P, McGill N, Hayward C, Firth H, Markham AF, Fantes JA, Bonthron DT: **Identification of SATB2 as the cleft palate gene on 2q32-q33.** *Hum Mol Genet* 2003, **12**(19):2491–2501.
21. Alkuraya FS, Saadi I, Lund JJ, Turbe-Doan A, Morton CC, Maas RL: **SUMO1 haploinsufficiency leads to cleft lip and palate.** *Science* 2006, **313**(5794):1751.
22. Benko S, Fantes JA, Amiel J, Kleinjan DJ, Thomas S, Ramsay J, Jamshidi N, Essafi A, Heaney S, Gordon CT, McBride D, Golzio C, Fisher M, Perry P, Abadie V, Ayuso C, Holder-Espinasse M, Kilpatrick N, Lees MM, Picard A, Temple IK, Thomas P, Vazquez MP, Vekemans M, Crollius HR, Hastie ND, Munnich A, Etchevers HC, Pelet A, Farlie PG, Fitzpatrick DR, Lyonnet S: **Highly conserved non-coding elements on either side of SOX9 associated with Pierre Robin sequence.** *Nat Genet* 2009, **41**(3):359–364.
23. Milunsky JM, Maher TA, Zhao G, Roberts AE, Stalker HJ, Zori RT, Burch MN, Clemens M, Mulliken JB, Smith R, Lin AE: **TFAP2A mutations result in branchio-oculo-facial syndrome.** *Am J Hum Genet* 2008, **82**(5):1171–1177.
24. Osoegawa K, Vessere GM, Utami KH, Mansilla MA, Johnson MK, Riley BM, L’Heureux J, Pfundt R, Staaf J, van der Vliet WA, Lidral AC, Schoenmakers EFPM, Borg A, Schutte BC, Lammer EJ, Murray JC, de Jong PJ: **Identification of novel candidate genes associated with cleft lip and palate using array comparative genomic hybridisation.** *J Med Genet* 2008, **45**(2):81–86.
25. Sivertsen A, Lie RT, Wilcox AJ, Abyholm F, Vindenes H, Haukanes BI, Houge G: **Prevalence of duplications and deletions of the 22q11 DiGeorge syndrome region in a population-based sample of infants with cleft palate.** *Am J Med Genet A* 2007, **143**(2):129–134.
26. Shi M, Mostowska A, Jugessur A, Johnson MK, Mansilla MA, Christensen K, Lie RT, Wilcox AJ, Murray JC: **Identification of microdeletions in candidate genes for cleft lip and/or palate.** *Birth Defects Res A Clin Mol Teratol* 2009, **85**:42–51.