ARTICLE

Genome-wide analysis of parent-of-origin effects in non-syndromic orofacial clefts

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Parent-of-origin (PofO) effects, such as imprinting are a phenomenon where the effect of variants depends on parental origin. Conventional association studies assume that phenotypic effects are independent of parental origin, and are thus severely underpowered to detect such non-Mendelian effects. Risk of orofacial clefts is influenced by genetic and environmental effects. the latter including maternal-specific factors such as perinatal smoking and folate intake. To identify variants showing PofO effects in orofacial clefts we have used a modification of the family-based transmission disequilibrium test to screen for biased transmission from mothers and fathers to affected offspring, biased ratios of maternal versus paternal transmission, and biased frequencies of reciprocal classes of heterozygotes among offspring. We applied these methods to analyze published genomewide single-nucleotide polymorphism (SNP) data from \sim 2500 trios mainly of European and Asian ethnicity with non-syndromic orofacial clefts, followed by analysis of 64 candidate SNPs in a replication cohort of \sim 1200 trios of European origin. In our combined analysis, we did not identify any SNPs achieving conventional genome-wide significance ($P < 5 \times 10^{-8}$). However, we observed an overall excess of loci showing maternal versus paternal transmission bias (P=0.013), and identified two loci that showed nominally significant effects in the same direction in both the discovery and replication cohorts, raising the potential for PofO effects. These include a possible maternal-specific transmission bias associated with rs12543318 at 8g21.3, a locus identified in a recent meta-analysis of non-syndromic cleft (maternal-specific $P = 1.5 imes 10^{-7}$, paternal-specific P = 0.17). Overall, we conclude from this analysis that there are subtle hints of PofO effects in orofacial clefting. European Journal of Human Genetics (2014) 22, 822-830; doi:10.1038/ejhg.2013.235; published online 30 October 2013

Keywords: maternal effect; folic acid; imprinting; cleft lip; cleft palate

INTRODUCTION

Orofacial clefting (OFC) is one of the most common of all human congenital birth defects with a prevalence rate of 1 in 700 live births worldwide.^{1,2} It may either occur in the context of malformation syndromes, or as an isolated anomaly (ie, non-syndromic OFC, NSOFC). Non-syndromic clefts are divided into two categories on the basis of epidemiological and embryological research findings: (i) cleft lip with or without cleft palate (CL/P) and (ii) cleft palate only (CPO).^{3,4}

Although genome-wide association studies (GWAS) have identified several genetic loci associated with risk oral clefting,^{5–7} there is significant evidence of other biological factors contributing to etiology of OFC.⁸ These may include parent-of-origin (PofO) effects such as imprinting,⁹ and other effects such as maternal–fetal interactions. In fact, environmental studies have indicated maternal smoking, and potentially alcohol consumption, during pregnancy greatly increase the risk of clefting in offspring, with some evidence of genetic interactions.^{2,10,11} Similarly, according to several studies, multi-vitamin supplements with or without folic acid taken during pregnancy have been shown to decrease the risk of oral clefting, with a stronger effect seen in CL/P as compared with CPO.^{12–14} If levels of

circulating folate are influenced by genetic factors, then it can be hypothesized any maternal genes involved in dictating circulating folate levels could also alter the risk of OFCs in the fetus. Indeed, evidence in support of this idea has recently been published.¹⁵ As such, some genetic effects on disease risk can operate via a mechanism of maternal/fetal interaction. Previous candidate gene studies have suggested evidence for PofO effects in CL/P providing a strong rationale for performing comprehensive analysis to identify unconventional modes of inheritance.^{16–19}

However, non-Mendelian inheritance patterns such as imprinting are generally ignored in conventional GWAS, as these tests ignore the differential effects of maternally and paternally inherited alleles on phenotype. Indeed, simple case–control GWAS are unable to address this question. A detailed assessment of the extent of PofO effects across the genome is therefore important for the proper understanding of genome function in relation to disease. Although individual candidate gene studies have suggested possible PofO effects in OFC, a recent GWAS specifically investigated the role of PofO effects in OFCs, but was unable to identify any loci showing genomewide significant effects.²⁰

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Previously, we used association methods specifically designed to detect PofO effects, and applied these to successfully detect quantitative trait loci exerting PofO effects on the expression of imprinted genes missed by conventional approaches.²¹ These methods take advantage of trio data to first define the parental origin of each allele in the offspring based on rules of Mendelian inheritance, and then perform separate association analyses of the two parental genotypes.

To analyze the role of PofO effects in common complex diseases, here we have employed an extension of the transmission asymmetry test and parental asymmetry test, to detect PofO effects using singlenucleotide polymorphism (SNP) genotyping in trios.^{22,23} We applied these methods to re-analyze publicly available genotype data from trios with NSOFC from the database of Genotypes and Phenotypes.²⁴ Our strategy analyzes associations separately for the maternally and paternally derived alleles, providing considerably increased power to detect PofO effects over conventional GWAS approaches. By first annotating parental origin of each allele in the affected offspring, we can conduct specific tests for association with maternal and paternal alleles independently, together with additional tests to look for differential effects of alleles inherited maternally versus paternally. From this initial genome-wide screen, we then selected 64 SNPs showing the strongest putative transmission bias for follow-up in a replication cohort of ~ 1200 additional trios of European origin. Although we failed to identify any loci with PofO affects achieving genome-wide significance, our results do provide evidence suggesting biases for maternally inherited genetic factors influencing the risk of NSOFC.

METHODS

Genome-wide detection of putative PofO effects

Genome-wide SNP data for 7018 individuals comprising 2339 trios in which each child was affected with any type of NSOFC (CL/P or CPO), were downloaded from dbGaP (Accession number: phs000094.v1.p1).7 Available genotype data included a total of 1387466 SNPs, comprising 601273 genotyped SNPs and an additional 786193 SNPs with discrete genotypes imputed by BEAGLE using HapMap Phase II samples as a reference panel. After converting high confidence imputed SNPs at $r^2 \ge 0.9$ to their respective genotypes and filtering non-informative SNPs (see Supplementary Information), we identified the transmitted and non-transmitted alleles in each parent, and the paternally and maternally inherited alleles in each child using rules of Mendelian inheritance. We then performed four different tests to detect putative PofO effects: (i) analysis of transmission bias from heterozygous fathers to affected children (PAT); (ii) analysis of transmission bias from heterozygous mothers to affected children (MAT); (iii) a comparison with the maternal and paternal odds ratios (PofO); and (iv) a comparison of the relative frequency of the two classes of heterozygotes in affected children (HET) (Figure 1). We analyzed this data set using seven different combinations based on disease subtype (NSCL/P and NSCPO^{3,4}) and ethnic groups (Europeans and Asians). The number of SNPs and samples used in each analysis are shown in Table 1.

SNPs showing putative parental-specific transmission bias in this discovery cohort were defined as follows: as a primary filter, we first selected those SNPs showing nominal significance ($P_{PofO} < 0.05$) in the PofO test, despite the large number of tests. Then, using a significance threshold of $P < 1 \times 10^{-5}$, SNPs were considered to show a possible PofO bias if they were significant in any of the four tests: PAT, MAT, PofO and HET. A subset of these SNPs was then carried forward for further investigation in a replication cohort (see below).

Using the PLINK -blocks function,²⁵ we calculated the number of distinct linkage disequilibrium (LD) blocks containing GWAS SNPs showing $P_{\text{PofO}} < 0.05$, and either $P_{\text{MAT}} < 10^{-4}$ or $P_{\text{PAT}} < 10^{-4}$. Enrichment analysis was performed using χ^2 -test with d.f. = 1, under the null hypothesis that there should be equal numbers of MAT and PAT LD blocks.

Replication study

We selected 64 SNPs (32 SNPs for NSOFC, 33 SNPs for NSCL/P and 5 SNPs for NSCPO; with six SNPs shared between NSOFC and NSCL/P) for a replication study using 1197 European trios. Seven hundred and forty six trios were part of the EUROCRAN/ITALCLEFT studies (273 from the Netherlands, 124 from Italy, 118 from the UK, 73 from Slovakia, 71 from Hungary, 33 from Bulgaria, 23 from Slovenia, 21 from Estonia and 10 from Spain), and the 451 remaining trios were recruited in Bonn, Germany.⁶ Two additional SNPs overlapping *SEMA4D*, a gene with roles in axon guidance,²⁶ were also included for CLP + CPO replication analysis. At the phenotypic level, the sample was subdivided into 931 trios where the index patient had NSCL/P and 266 with NSCPO (see Supplementary Information).

Genotyping was conducted using Sequenom MALDI-ToF mass spectrometer MassArray system (Sequenom Inc., San Diego, CA, USA). Primers were synthesized at Metabion (Martinsried, Germany). Using Sequenom MassARRAY Assay Design Software 3.4, two multiplex assays comprising all 64 selected SNPs plus the gender-specific variant were designed. Genotype data were analyzed using Sequenom Spectrodesigner Software package. Inter- and intraplate duplicates were included to check for genotype consistencies across DNA plates. Allele peaks were analyzed using Sequenom Typer Analysis software and genotype calls were confirmed by visual inspection of cluster plots. After quality control filtering, our final filtered replication data set comprised 48 SNPs mapping to 38 distinct chromosomal loci (see Supplementary Information). As the patient consent does not allow any unrestricted release of data, even in anonymised form, genotypes were not deposited in a public database. However, genotypes can be provided by the authors upon request.

In the replication sample PAT, MAT, PofO and HET tests for analysis of PofO effects were performed, as described above. Subsequently, combined analyses in which we pooled both the discovery and replication samples were conducted.

RESULTS

Genome-wide analysis of PofO effects

Table 1 summarizes the results for genome-wide analysis of PofO effects in the different phenotypic and ethnic groups analyzed. Based on a low stringency discovery threshold of $P < 1 \times 10^{-5}$ in any of the four tests for PofO effects (PAT, MAT, PofO and HET), among the combined NSCL/P and NSCPO samples, we identified a total of 55 SNPs (representing 13 distinct LD blocks) when the two ethnic groups were analyzed together, 31 SNPs (21 LD blocks) using only European samples, and 9 SNPs (8 LD blocks) using only Asian samples (Supplementary Table 1). Similarly, when performing our analysis based on each disease subtype, we identified an additional 52, 21 and 16 SNPs (falling in 14, 17 and 12 separate LD blocks) associated with NSCL/P based on the analysis of all samples combined (Europeans and Asians), respectively (Supplementary Table 2). In the analysis of NSCPO, we identified 36 SNPs (representing 19 LD blocks) when analyzing all ethnic groups (Supplementary Table 3).

The most significant site, showing a putative association only when transmitted by the mother, was produced by SNP rs3814878 (16p11.2) in the MAT test ($P_{MAT} = 1.69 \times 10^{-7}$). This same SNP showed no significant transmission bias in PAT test ($P_{PAT} = 0.052$) (Figure 2, SNP M11). This region contains an interesting candidate gene for OFC, namely *TBX6* (Supplementary Figure 1).

To investigate whether there was any bias in the transmission ratio of nominally significant associations between the two parental alleles, we calculated the number of independent loci (LD blocks) containing any SNP exceeding a threshold of $P < 1 \times 10^{-4}$ in the MAT and PAT tests for each ethnic group and each category of OFC (Figure 3). In every case the total number of maternal-specific signals equaled or exceeded the number of paternal-specific signals, with a significant excess of maternal-specific signals observed in three categories: All

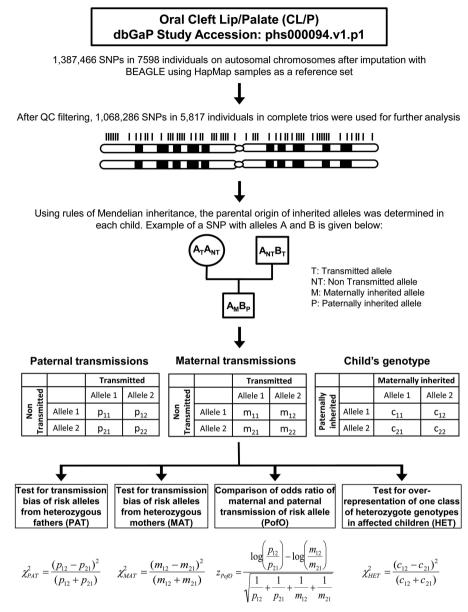


Figure 1 Summary of the method used to screen for parent-of-origin effects in orofacial clefts.

(NSCL/P + NSCPO, P = 0.013), Europeans (NSCL/P + NSCPO, P = 0.026) and All (NSCPO, P = 0.003). A similar excess of loci showing maternal-specific transmission bias was also observed using SNPs with an increased significance threshold of $P < 1 \times 10^{-5}$ in the MAT and PAT tests, although due to the smaller number of loci, these differences did not reach statistical significance (data not shown).

We calculated the genomic inflation factors for the MAT and PAT tests (Supplementary Table 4). We observed a small increase in the genomic inflation factor for the MAT compared with the PAT in all categories, although the magnitude of this bias is small, with the maximum genomic inflation factor observed being 1.04. QQ plots for the MAT and PAT are shown in Supplementary Figure 2.

Replication study

The results of replication analysis using 48 SNPs passing all quality filtering steps are summarized in Table 2 and Supplementary Table 5.

Among the SNPs exceeding our low stringency discovery thresholds in the initial GWAS ($P \le 1 \times 10^{-5}$), only two passed our thresholds for successful replication, showing $P \le 0.01$ with the MAT or PAT in the replication cohort in the same direction as observed in the discovery phase. Considering the replication data alone, the most significant P-value observed was $P_{\text{PAT}} = 0.002$ at SNP rs719325 in NSCL/P (Figure 4). This same SNP yielded $P_{\text{PAT}} = 8.1 \times 10^{-6}$ in the discovery GWAS (using combined European and Asian samples), giving a combined $P_{\text{PAT}} = 5.4 \times 10^{-8}$. However, although in the discovery GWAS this SNP yielded $P_{PofO} = 0.005$ and $P_{MAT} = 0.69$ suggesting this locus shows a paternal-specific transmission bias, this effect was not seen in the replication cohort, with $P_{PofO} = 0.41$ and $P_{\text{MAT}} = 0.048$. Results using a conventional TDT test for this SNP were $P_{\text{TDT}} = 7.8 \times 10^{-4}$ in the discovery GWAS, $P_{\text{TDT}} = 0.002$ in the replication cohort, yielding a combined $P = 4.9 \times 10^{-6}$. A second SNP rs12543318, yielded $P_{MAT} = 2.2 \times 10^{-6}$ in the discovery GWAS (NSOFC in European samples), and $P_{MAT} = 0.004$ in the replication

Table 1 Summary of genome-wide screening for parent-of-origin effects in oral clefts

Cleft type	Ethnicity	Number of complete trios utilized in genome-wide analysis	Number of filtered SNPs used in genome-wide analysis	No. of SNPs (independent LD blocks) identified in genome-wide screen with $P < 1 \times 10^{-5}$ in MAT, PAT, PofO or HET tests	SNPs carried for- ward to replication study
NSCL/P and NSCPO	All individuals	1939	1 068 286	55 (13)	11+2 ^a
	Europeans	758	1 060 874	31 (21)	19
	Asians	949	952 138	9 (8)	—
NSCL/P	All individuals	1491	1068429	52 (14)	15
	Europeans	570	1 060 675	21 (17)	18
	Asians	742	952438	16 (12)	_
NSCPO	All individuals	437	1 068 897	36 (19)	5

Abbreviations: HET, heterozygous; LD, linkage disequilibrium; MAT, maternal; NSCL/P, non-syndromic cleft lip with or without cleft palate; NSCPO, non-syndromic cleft palate only; PAT, paternal; PofO, parent-of-origin; SNP, single-nucleotide polymorphism.

^aTwo SNPs overlapping the SEMA4D gene were added to our replication set (see Methods).

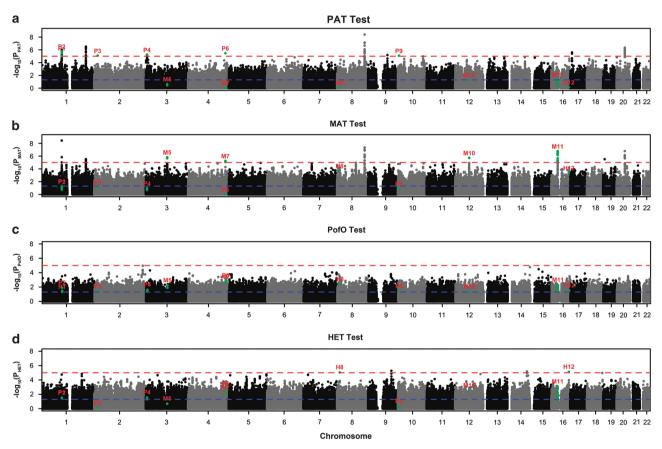


Figure 2 Manhattan plot showing the results of four tests for 22 autosomes performed using all ethnic groups (Europeans and Asians) and disease subtypes (CL/P and CPO) combined. The y axis shows the $-\log_{10}$ P-value for each test and the red and blue lines represent thresholds of $P < 1 \times 10^{-5}$ (suggestive association) and 0.05 (nominal significance), respectively. Green dots represent loci showing suggestive associations identified by the four tests (see Methods). Each locus is labeled based on the test showing P<1×10⁻⁵: P# for PAT, M# for MAT, PofO# for PofO test and H# for HET. The P-values generated by four tests for each locus are shown in all four panels using the same label. For example, all SNPs at M11 loci have P<10⁻⁵ in MAT (above red line), while the same SNPs show P>0.05 in PAT (below blue line). These SNPs show nominal significance in PofO and HET (between blue and red line).

cohort, giving a combined $P_{MAT} = 1.5 \times 10^{-7}$ (Figure 4). However, although this SNP yielded $P_{PofO} = 0.009$ in the GWAS PofO test, and $P_{\text{PAT}} = 0.24$ and 0.43 in the discovery and replication cohorts, respectively, suggesting a putative maternal-specific transmission bias, results for the PofO test in the replication cohort were not clear (P = 0.12). Results using a conventional TDT test for this SNP were $P_{\text{TDT}} = 1.2 \times 10^{-4}$ in the discovery GWAS, $P_{\text{TDT}} = 0.022$ in the replication cohort, yielding a combined $P_{\text{TDT}} = 2.4 \times 10^{-5}$. This same SNP also yielded very similar results in NSCL/P using both European alone, and the combined European and Asian GWAS and replication samples.

Results for the 48 SNPs using a conventional TDT (which does not consider parental origin) are listed in Supplementary Table 6. We observed four SNPs (corresponding to three LD blocks) yielding both

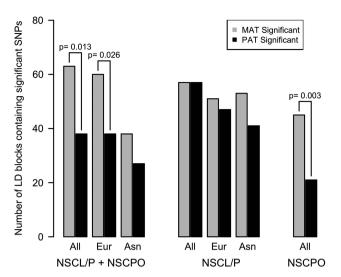


Figure 3 A global bias for maternal genetic effects in the etiology of OFCs. In every comparison using different subtypes of orofacial clefts and ethnicities (All: all ethnic groups, Eur: Europeans and Asn: Asians), we observed that the number of maternal-specific signals equals or exceeds the number of paternal-specific signals. A significant excess (*P*<0.05) of maternal-specific signals is observed in three categories, as indicated. Bar plots show the number of independent loci (LD blocks) with *P*<1 × 10⁻⁴ identified using the MAT (gray) and PAT (black) tests. A similar excess of maternal associations was also observed using a more stringent significance threshold of *P*<10⁻⁵, although due to the smaller number of loci, these differences did not reach statistical significance (data not shown).

 P_{TDT} < 0.01 in the discovery cohort and P_{TDT} < 0.05 in the replication cohort.

DISCUSSION

In this study, we have applied modified versions of the Transmission Asymmetry Test and Parental Asymmetry Test to perform genomewide screening for PofO effects associated with non-syndromic oral cleft in mother/father/affected child trios. We used a previously published genome-wide SNP data from ~2500 trios for discovery,⁷ followed by genotyping of selected SNPs in a replication cohort of ~1200 trios. Using four tests (PAT, MAT, PofO and HET – see Methods), a low stringency threshold in the discovery cohort identified a total of 210 SNPs (corresponding to 88 independent regions) showing evidence of possible parental-specific transmission bias associated with risk of non-syndromic oral clefts (NSCL/P or NSCPO) in various ethnic groups (all, European or Asian populations). Of these discovery SNPs, 64 were carried forward for testing in the replication cohort.

Although in our replication analysis we observed several loci reaching nominal significance in the same direction as the discovery GWAS data, none of the markers tested achieved generally accepted definitions of genome-wide significance ($P < 5 \times 10^{-8}$) in the combined sample. However, there are several possible explanations for failure to replicate GWAS signals, including the 'winners curse' phenomenon.²⁷ Of particular note, our replication cohort was approximately half the size of our discovery cohort, and as a result is underpowered to detect small effects on disease risk detected in our discovery phase.

We identified two loci that passed our thresholds for successful replication $(P \le 1 \times 10^{-5}$ in the discovery cohort and $P \le 0.01$ in replication with either the MAT or PAT), raising the potential for PofO effects tagged by these loci. Paternally biased transmission was

detected for rs719325 at 2q25, and this result approached genomewide significance in the combined analysis ($P_{PAT} = 5.4 \times 10^{-8}$). This marker is located ~164 kb upstream of *SLC4A3*, a gene which encodes a trans-membrane transport protein involved in regulation of intracellular pH. *SLC4A3* has been described as a candidate gene for human retinal degeneration,²⁸ however, no role in craniofacial development has been described so far. Aside from genomic imprinting, mechanistically it is harder to conceptualize how paternal-specific effects on disease risk might operate, although some have been recognized.⁹

We also identified a possible maternal-specific transmission bias associated with rs12543318 located within 8q21.3. This same SNP was recently identified as a susceptibility locus for NSCL/P in a recent meta-analysis.²⁹ This marker maps to an intergenic region for which, so far, no functional information related to OFC is available. Although the meta-analysis of Ludwig et al.²⁹ and the current study utilized many of the same individuals, this previous study did not test for PofO effects. Comparison of the TDT results for the NSCL/P group in the present and the trio-part of Ludwig et al²⁹ reveals they are in the same range (with the slight difference being attributed to different quality controls on samples). However, the present PofO analysis suggests the signal at rs12543318 is largely attributable to maternally derived alleles, as our MAT test yielded a combined $P = 1.5 \times 10^{-7}$, while the corresponding PAT test gave a combined P = 0.17, with a combined $P_{PofO} = 0.004$. No known imprinting effects map to this region. Thus, the 8q21.3 region might contain a genetic element which in some way interacts with other maternalspecific risk effects.

Two other loci that achieved suggestive evidence of replication $(P \le 1 \times 10^{-5} \text{ in discovery and } P \le 0.1 \text{ in replication with the MAT})$ are worthy of mention. rs17447439 maps within an intron of the TP63 gene at 3q28, and showed weak evidence of a maternal-specific transmission bias. Several studies provide evidence linking TP63 with the development of OFC. For example, a homozygous mutation in TP63 has been suggested to have a causative role in NSCL/P.30 Further, a recent genome-wide analysis of p63-binding sites identified the AP-2a transcription factor as target site.³¹ AP-2a is known to be essential for craniofacial development and cranial closure³² and has been implemented in NSCL/P.³³ Similarly in the analysis of NSCPO, a suggestive bias for maternal over-transmission was observed for rs6539608 located on chromosome 12q21. No genes or transcripts map to this region, making an interpretation of functional relevance difficult. Notably, however, Koillinen et al34 found suggestive linkage to this locus in a study comprising nine Finnish multiplex families affected with NSCPO.34

On balance our analysis finds only weak evidence for specific genetic loci individually contributing to PofO effects in OFC. After performing replication analysis of 48 SNPs showing suggestive evidence of PofO effects in the discovery cohort, individually none of these showed unambiguous confirmation of statistical signals that would allow us to define any PofO effects with confidence. These results are broadly consistent with the study of Shi *et al.*²⁰ which utilized an alternative method to analyze the same discovery cohort. One of the limitations of our method compared with the method used in Shi et al20 is the inability to differentiate between different types of parental biases, such as imprinting or maternal effects. Our analysis approach also does not directly consider environmental effects such as the maternal intrauterine environment. One advantage of our method, however, is that by performing independent tests of the maternally and paternally derived alleles (MAT and PAT tests) we are able to directly compare the relative

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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	SNPs significant	in NSOFC in the	e European GWAS sar	mple analysis											
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Chr2 3154990 0.287 2.81E.06 0.015 0.070 0.255 0.170 0.873 0.738 0.121 1.24 Chr2 88937456 0.237 2.81E.06 0.003 0.003 0.016 0.317 0.005 0.011 Chr3 1836873 5.57E.06 0.138 0.003 0.016 0.317 0.005 0.011 0.126 0.024 0.126 0.024 0.126 0.024 0.126 0.024 0.126 0.025 0.001 0.015 0.317 0.005 0.011 0.027 0.024 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.023 0.021 0.021 0.021 0.021 0.021 0.021 <th0.021< th=""> 0.023 0.021</th0.021<>	s4925671	Chr1	245689497	0.816	5.65E-06	0.002	0.005	0.034	0.458	0.043	0.626	0.158	3.87E-04	4.36E-04	0.026
Chrd 17385723 5 67F-06 0.731 0.003 0.144 0.110 0.560 0.405 0.011 0.114 0.115 0.125 0.126 0.127 0.126 0.127 0.126 0.127 0.126 0.013 <th0.013< th=""> 0.013 0.013</th0.013<>	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	s10208086	Chr2	3154999	0.287	2.81E-06	0.015	0.070	0.255	0.170	0.873	0.738	0.121	4.57E-05	0.085	0.166
CHG B8937456 0.237 2.4E-06 0.003 0.012 0.012 0.166 0.23 0.012 0.012 0.032 <th< td=""><td>$\begin{array}{cccccccccccccccccccccccccccccccccccc$</td><td>s820042</td><td>Chr2</td><td>173853723</td><td>5.67E-06</td><td>0.751</td><td>0.003</td><td>0.008</td><td>0.414</td><td>0.110</td><td>0.560</td><td>0.405</td><td>0.001</td><td>0.147</td><td>0.166</td><td>0.410</td></th<>	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	s820042	Chr2	173853723	5.67E-06	0.751	0.003	0.008	0.414	0.110	0.560	0.405	0.001	0.147	0.166	0.410
CH6 110900618 0.015 0.044 6.616.06 0.209 0.031 0.0905 0.722 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.792 0.001 0.732 0.002 0.035 0.732 0.002 0.035 0.112 0.755 0.113 0.002 0.002 0.002 0.002 0.002 0.002 0.002 0.001 0.023 0.011 <th0.011< th=""> 0.011 0.011</th0.011<>	(10) (10)	s12543318	Chr8	88937456	0.237	2.24E-06	0.009	0.109	0.433	0.004	0.122	0.462	0.174	1.54E-07	0.004	0.106
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	s1519466	Chr9	110900618	0.198	0.075	0.034	6.61E-06	0.209	0.030	0.016	0.317	0.905	0.792	0.792	0.019
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	s12264639	Chr10	15368599	0.034	3.44E-05	9.46E-06	0.004	0.386	0.257	0.156	0.024	0.523	0.085	0.091	0.968
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	s1533763	Chr11	75578175	0.630	7.66E-06	3.41E-04	0.002	0.411	0.695	0.768	0.620	0.732	0.002	0.050	0.129
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	s4944925	Chr11	74037177	0.082	0.022	0.004	5.97E-06	0.817	0.275	0.351	0.765	0.189	0.022	0.011	0.001
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		s927053	Chr14	58524433	0.783	6.11E-06	0.001	0.535	0.932	0.697	0.841	0.125	0.901	0.014	0.100	0.473
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		s7173522	Chr15	99135612	2.21E-06	0.141	0.025	0.008	0.530	0.107	0.461	0.788	0.001	0.029	0.417	0.057
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Chr20 5343077 0.382 $7.24E.06$ 0.010 0.056 0.387 0.274 0.865 0.438 0.899 0.023 ant in NSCLP in the combined European and Aisin GMAS sample analysis 0.002 0.002 0.048 0.112 0.403 $5.43E.08$ 0.012 0.432 0.326 0.335 0.002 0.489 0.322 0.336 0.012 0.902 0.112 0.448 0.312 0.326 0.012 0.026 0.026 <td>s11644607</td> <td>Chr16</td> <td>74806998</td> <td>0.004</td> <td>0.905</td> <td>0.028</td> <td>1.97E-07</td> <td>0.061</td> <td>1.000</td> <td>0.172</td> <td>0.095</td> <td>0.001</td> <td>0.937</td> <td>0.013</td> <td>1.72E-06</td>	s11644607	Chr16	74806998	0.004	0.905	0.028	1.97E-07	0.061	1.000	0.172	0.095	0.001	0.937	0.013	1.72E-06
art in NSC/F in the combined European and Asian GMMS sample analysis art in NSC/F in the combined European and Asian GMMS sample analysis Chr3 6194615 2.24E-06 0.654 0.003 0.066 0.448 0.412 0.403 6.018 0.067 Chr3 6194615 2.24E-06 0.654 0.003 0.060 0.489 0.392 0.356 0.012 0.918 0.067 Chr3 101148211 0.109 4.14E-07 0.016 0.119 0.326 0.022 0.335 0.032 0.033 0.035 0.326 0.012 0.918 0.004 Chr12 910035832 0.0105 4.34E-06 0.016 0.116 0.226 0.775 0.935 0.002 1.67E-05 0.233 Chr12 91032110 0.119 0.34E-06 0.117 0.248 0.010 0.124 0.326 0.007 0.326 0.026 0.326 0.026 0.326 0.026 0.326 0.026 0.326 0.026	ant in NSCLP in the combined European and Asian GMAS sample analysis chr2 203376177 8.07E-06 0.005 0.005 0.013 0.013 5.43E-08 0.013 0.013 5.43E-08 0.012 0.013 5.43E-08 0.012 0.013 5.43E-08 0.012 0.012 <th< td=""><td>s3852922</td><td>Chr20</td><td>53443077</td><td>0.382</td><td>7.24E-06</td><td>0.010</td><td>0.056</td><td>0.387</td><td>0.274</td><td>0.865</td><td>0.438</td><td>0.899</td><td>0.046</td><td>0.133</td><td>0.525</td></th<>	s3852922	Chr20	53443077	0.382	7.24E-06	0.010	0.056	0.387	0.274	0.865	0.438	0.899	0.046	0.133	0.525
Chr2 Constrained for the formation of the formatio	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	SNPs significant	in NSCL/P in the	e comhined Furnhear	r and Asian GW	AS cample ana	lucic									
1000000000000000000000000000000000000	Child Z2007011 0.002 0.003 0.003 0.003 0.003 0.004 0.4412 0.4412 0.4403 0.4412 0.4403 0.4412 0.4403 0.4413 0.4403 0.4413 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 0.012 0.013 <td>-71037E</td> <td></td> <td>2000212122</td> <td>O D TE DE</td> <td></td> <td></td> <td>0 262</td> <td></td> <td>0100</td> <td>0110</td> <td></td> <td>E 12E 00</td> <td>0 1 1 0</td> <td>200.0</td> <td>3000</td>	-71037E		2000212122	O D TE DE			0 262		0100	0110		E 12E 00	0 1 1 0	200.0	3000
Und 10059812 $2.242-00$ 0.003 0.003 0.004 0.004 0.001 0.0112 0.0112 0.0112 0.0112 0.0012 0	Cm3 0134013 $2.24\pm.06$ 0.003 0.003 0.003 0.003 0.003 0.012 0.003 0.012 0.003 0.012 0.003 0.012 0.003 0.012 0.003 0.012 0.013 0.012 0.012 0.013 0.012 0.010 0.012 0.010 0.012 0.010 0.012 0.010 0.012 0.010 0.012 0.010 0.012 0.010 0.012 0.010 0.012 0.010 0.010 0.010 0.010 0.010 0.012 <t< td=""><td>S/ 19363</td><td></td><td>// 10/0077</td><td>0.0/E-00</td><td>0.092</td><td>0.00</td><td>0.000</td><td>0.00</td><td>0.040</td><td>0.412</td><td></td><td>0.43E-00</td><td>0.110</td><td>0.000</td><td>0.00.0</td></t<>	S/ 19363		// 10/0077	0.0/E-00	0.092	0.00	0.000	0.00	0.040	0.412		0.43E-00	0.110	0.000	0.00.0
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Christ 100995832 0.018 3.30 -0 0.119 0.960 0.1218 0.304 0.906 0.282 0.009 0.282 0.000 0.244 0.965 0.024 33 0.01148211 0.101 $4.14E-07$ 0.016 0.116 0.326 0.727 0.336 0.010 1.313 0.336 0.010 1.311 0.336 0.010 1.311 0.320 0.010 1.311 0.320 0.010 0.336 0.010 0.322 0.161 0.010 0.322 0.161 <th0< th=""> 0.026 0.026</th0<>	s/618286	Chr3	6194615	2.24E-U6	0.654	0.003	0.003	0.060	0.489	0.392	0.356	210.0	0.918	0.067	0.004
Christ 101148211 0.109 4.14E-07 0.016 0.196 0.110 0.262 0.751 0.995 0.024 385E-06 0.094 Christ 8191032117 0.191 6.94E-06 0.049 0.001 1.000 0.662 0.202 0.131 0.320 2.63E-06 0.047 Christ 91938211 0.191 6.94E-06 0.147 0.033 0.031 0.481 0.032 0.320 2.63E-06 0.047 Christ 94789821 0.269 9.20E-06 9.44E-05 0.001 3.36E-06 0.389 0.022 0.342 0.385-06 0.007 Christ 119645972 0.059 9.147 0.031 0.341 0.385-06 0.007 Christ 119645972 0.059 9.133 0.040 9.722 0.161 0.200 0.667 Christ 11964507 0.337 9.056-06 0.313 0.040 0.522 0.151 0.200 0.067 Christ 82206283	Christ 101148211 0.109 $4.14E-07$ 0.016 0.196 0.110 0.262 0.757 0.955 0.024 33 Christ 19103211 0.191 6.94E-06 0.049 0.001 1.000 0.062 0.207 0.354 0.336 0.010 Christ 988937456 0.005 4.30E-06 0.147 0.033 0.031 0.143 0.324 0.33 0.010 1 0.320 0.010 1 0.320 0.010 1 0.326 0.548 0.010 1 0.320 0.010 1 0.320 0.010 1 0.320 0.010 1 0.320 0.010 1 0.326 0.583 0.0124 33 0.010 1 0.320 0.016 0.010 1 0.320 0.010 1 0.010 0.116 0.011 0.010 0.011 0.010 0.011 0.010 0.011 0.010 0.012 0.016 0.016 0.016 0.016 0.016 0.016 <td>s/045/0</td> <td>Chr3</td> <td>75866600T</td> <td>0.018</td> <td>3.30E-06</td> <td>0.119</td> <td>0.960</td> <td>0.218</td> <td>0.304</td> <td>0.906</td> <td>7.82.0</td> <td>0.009</td> <td>I.6/E-U5</td> <td>0.233</td> <td>0.462</td>	s/045/0	Chr3	75866600T	0.018	3.30E-06	0.119	0.960	0.218	0.304	0.906	7.82.0	0.009	I.6/E-U5	0.233	0.462
Chris 191032117 0.191 $6.94F.06$ 0.049 0.001 1.000 0.062 0.220 0.131 0.320 2.65F.06 0.016 Chril 91869646 5.40E-06 0.147 0.033 0.021 0.481 0.092 0.206 0.942 0.007 Chril 91869646 5.40E-06 0.147 0.033 0.031 0.481 0.092 0.206 7.90E-05 0.942 0.007 Chril2 91964597 0.029 9.24E-06 0.313 0.021 0.883 0.202 0.161 0.020 0.342 0.007 Chril2 11964597 0.029 0.313 0.044 0.582 0.722 0.881 0.081 0.166 0.056 Chril2 11964597 0.029 0.312 0.1266 0.326 0.126 0.320 0.166 0.056 0.366 0.316 0.001 1.80E-07 0.071 Chrl3 7741898 0.056 0.320 1.26E-04 0.009 0.152	Chr3 191032117 0.191 $6.94E \cdot 06$ 0.001 1.000 0.062 0.202 0.131 0.320 2 Chr11 91869646 $5.40E \cdot 06$ 0.147 0.033 0.031 0.481 0.092 0.206 7.90E \cdot 05 0.100 1.143 0.336 0.010 1 Chr12 94798821 0.269 9.20E \cdot 06 0.84E \cdot 05 0.001 3.36E \cdot 06 0.343 0.010 1.200 7.90E \cdot 05 0.206 7.90E \cdot 05 0.010 1.200 0.143 0.206 7.90E \cdot 05 0.010 1.200 0.0143 0.010 0.143 0.206 0.206 7.90E \cdot 05 0.010 1.200 0.206 0.206 7.90E \cdot 05 0.010 1.200 0.143 0.206 0.206 7.90E \cdot 05 0.010 1.200 0.141 0.202 0.161 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 0.206 <td>s13317017</td> <td>Chr3</td> <td>101148211</td> <td>0.109</td> <td>4.14E-07</td> <td>0.016</td> <td>0.196</td> <td>0.110</td> <td>0.262</td> <td>0.757</td> <td>0.955</td> <td>0.024</td> <td>3.85E-06</td> <td>0.094</td> <td>0.319</td>	s13317017	Chr3	101148211	0.109	4.14E-07	0.016	0.196	0.110	0.262	0.757	0.955	0.024	3.85E-06	0.094	0.319
Christ 88937456 0.005 $4.30E-06$ 0.176 0.207 0.548 0.010 0.143 0.336 0.010 1.80E-07 0.047 0.047 Chr11 91869646 5.40E-06 0.147 0.033 0.031 0.481 0.098 0.092 0.342 0.007 Chr12 91869646 5.40E-06 0.147 0.033 0.031 0.481 0.098 0.092 0.202 0.942 0.007 Chr12 119645972 0.059 9.20E-06 9.84E-05 0.001 $3.36E-06$ 0.833 0.722 0.161 0.200 0.365 Chr13 77441896 0.059 0.320 1.20E-04 0.004 0.520 0.899 0.203 0.166 0.842 0.166 Chr14 82206283 7.58E-06 0.139 1.20E-04 0.004 0.538 0.001 Failed QC in replication Chr20 61209037 9.05E-06 0.424 0.004 0.002 0.340 0.166 0.894 0.601	Chris 88937456 0.005 $4.30E-06$ 0.176 0.207 0.548 0.010 0.143 0.336 0.010 1.336 0.010 1.336 0.010 1.336 0.010 1.336 0.010 1.336 0.010 0.143 0.336 0.002 $7.90E-05$ 0.010 0.143 0.031 0.0481 0.002 0.141 0.0313 0.001 $3.36E-06$ 0.583 0.022 0.206 0.236 0.230 0.141 0.0313 0.001 $3.36E-06$ 0.2894 0.2894 0.230 0.161 0.081 0.081 0.081 0.081 0.011 0.081 0.081 0.081 0.081 0.081 0.081 0.081 0.081 0.081 0.081 0.010 0.161 0.0110 0.0110 0.1161 0.010 0.0112 0.0101 0.021 0.0101 0.0110 0.0111 0.0122 0.0111 0.0122 0.0011 0.0122 0.0111	s17447439	Chr3	191032117	0.191	6.94E-06	0.049	0.001	1.000	0.062	0.202	0.131	0.320	2.63E-06	0.016	4.61E-04
Chr11 91869646 5.40E-06 0.147 0.033 0.031 0.481 0.092 0.206 7.90E-05 0.942 0.007 Chr12 94798821 0.269 9.20E-06 9.84E-05 0.001 3.36E-06 0.583 0.729 0.899 0.222 0.161 0.200 0.058 Chr13 77441896 0.059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.061 0.058 Chr13 77441896 0.059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.061 0.342 0.001 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.081 0.370 0.001 Chr20 61209037 9.05E-06 0.320 1.20E-04 0.004 0.520 0.370 0.316 0.370 0.001 Chr20 61209037 9.05E-06 0.424 0.004 0.648 0.402	Chrl1 91869646 5.40E-06 0.147 0.033 0.031 0.481 0.092 0.206 $7.90E-05$ 0.01 Chrl2 94798821 0.269 9.20E-06 9.84E-05 0.001 $3.36E-06$ 0.583 0.729 0.899 0.222 0.161 0.01 Chrl2 119645972 0.002 0.149 0.001 $3.36E-06$ 0.583 0.729 0.899 0.222 0.161 0.081 0.01 0.0172 0.161 0.011 0.0172 0.161 0.011 0.0172 0.161 0.081 0.021 0.011 0.022 0.1161 0.021 0.011 0.0172 0.011 0.0172 0.1161 0.0222 0.1161 0.020 0.011 0.021 0.021 0.021 0.021 0.011 0.021 0.021 0.021 0.021 0.011 0.021 0.011 0.021 0.021 0.011 0.021 0.021 0.011 0.021	s12543318	Chr8	88937456	0.005	4.30E-06	0.176	0.207	0.548	0.010	0.143	0.336	0.010	1.80E-07	0.047	0.113
Chr12 94798821 0.269 9.20E-06 9.84E-05 0.001 Failed QC in replication Failed QC in replication Chr12 119645972 0.002 0.149 0.001 3.36E-06 0.583 0.729 0.899 0.222 0.161 0.200 0.058 Chr13 77441896 0.059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.061 0.200 0.058 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.081 0.370 0.001 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.081 0.370 0.001 Chr20 61209037 9.05E-06 0.424 0.003 0.139 Failed QC in replication and in NSCL/P in the European GWAS sample analysis 0.046 0.648 0.467 0.648 0.402 0.604 0.026 0.576 0.576 0.333	Chr12 94798821 0.269 9.20E-06 9.84E-05 0.001 Failed QC in replication Chr12 119645972 0.002 0.149 0.001 $3.36E-06$ 0.583 0.729 0.899 0.222 0.161 0 Chr13 77441896 0.0059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.081 0 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 Chr20 61209037 9.05E-06 0.320 1.20E-04 0.004 0.520 0.894 0.080 5.11E-05 0 and in NSCL/P in the European GWAS sample analysis failed QC in replication 0.041 0.467 0.648 0.402 0.604 0.026 0	s543704	Chr11	91869646	5.40E-06	0.147	0.033	0.031	0.481	0.098	0.092	0.206	7.90E-05	0.942	0.007	0.014
Chr12 119645972 0.002 0.149 0.001 3.36E-06 0.583 0.729 0.899 0.222 0.161 0.200 0.058 Chr13 77441896 0.059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.061 0.642 0.166 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.081 0.370 0.001 Chr20 61209037 9.05E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.081 0.370 0.001 Chr20 61209037 9.05E-06 0.424 0.009 0.139 Failed QC in replication and in NSCL/P in the European GWAS sample analysis 0.146 0.648 0.402 0.604 0.035 0.233 Chr1 161092955 7.86E-06 0.024 0.035 0.243 0.466 0.6164 0.026 0.233 Chr2 3154999 0.233 3.31E-06 </td <td>Chr12 119645972 0.002 0.149 0.001 $3.36E-06$ 0.583 0.729 0.899 0.222 0.161 0 Chr13 77441896 0.059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.081 0 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 Chr20 61209037 9.05E-06 0.424 0.004 0.139 746 0.588 0.080 5.11E-05 0 ant in NSCLP in the European GWAS sample analysis 7446 0.041 0.467 0.648 0.402 0.604 0.026 0.614 0.026 0.024 0.026 0.614 0.026 0.614 0.026 0.615 0.614 0.0224 0.0224<td>s2196457</td><td>Chr12</td><td>94798821</td><td>0.269</td><td>9.20E-06</td><td>9.84E-05</td><td>0.001</td><td></td><td>Failed QC in</td><td>· replication</td><td></td><td></td><td>Failed QC ir</td><td>n replication</td><td></td></td>	Chr12 119645972 0.002 0.149 0.001 $3.36E-06$ 0.583 0.729 0.899 0.222 0.161 0 Chr13 77441896 0.059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.081 0 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 Chr20 61209037 9.05E-06 0.424 0.004 0.139 746 0.588 0.080 5.11E-05 0 ant in NSCLP in the European GWAS sample analysis 7446 0.041 0.467 0.648 0.402 0.604 0.026 0.614 0.026 0.024 0.026 0.614 0.026 0.614 0.026 0.615 0.614 0.0224 0.0224 <td>s2196457</td> <td>Chr12</td> <td>94798821</td> <td>0.269</td> <td>9.20E-06</td> <td>9.84E-05</td> <td>0.001</td> <td></td> <td>Failed QC in</td> <td>· replication</td> <td></td> <td></td> <td>Failed QC ir</td> <td>n replication</td> <td></td>	s2196457	Chr12	94798821	0.269	9.20E-06	9.84E-05	0.001		Failed QC in	· replication			Failed QC ir	n replication	
Chr13 77441896 0.059 0.313 0.040 9.94E-06 0.812 0.172 0.420 0.388 0.081 0.842 0.166 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.081 0.370 0.001 Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0.370 0.001 Chr20 61209037 9.05E-06 0.424 0.009 0.139 Failed QC in replication Failed QC in replication ant in NSCL/P in the European GWAS sample analysis 0.746 0.003 0.041 0.467 0.648 0.402 0.576 0.233 Chr1 161092955 7.86E-06 0.746 0.035 0.243 0.166 0.914 0.095 6.31E-05 0.111 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.196 0.914 0.095 6.31E-05 0.111 Chr2 103247757 0.633 3.31E-06 0.007 0	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	s2948150	Chr12	119645972	0.002	0.149	0.001	3.36E-06	0.583	0.729	0.899	0.222	0.161	0.200	0.058	0.035
Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0.370 0.001 and in NSCL/P in the European GWAS sample analysis 0.424 0.009 0.139 Failed QC in replication Failed QC in replication Failed QC in replication Failed QC in replication and in NSCL/P in the European GWAS sample analysis 0.746 0.003 0.041 0.467 0.648 0.402 0.576 0.233 Chr1 161092955 7.86E-06 0.746 0.035 0.243 0.166 0.914 0.095 6.31E-05 0.111 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.995 6.31E-05 0.111 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.732 0.199 0.615 0.004 0.040 Chr5 4959824 0.731 7.875 0.113 0.855 0.004 0.040 0.040 Chr5 7666316 <td>Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 and in NSCL/P in the European GWAS sample analysis 0.424 0.009 0.139 Failed QC in replication 0.11E-05 0 and in NSCL/P in the European GWAS sample analysis 0.424 0.003 0.041 0.467 0.648 0.402 0.604 0.026 0 Chr1 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.604 0.026 0 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.914 0.095 6 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.6155 0 Chr3 103247757 0.633 3.31E-06 0.007 0.0355 0.559 0.853 0.899 0.962 0 Chr5 76663192 <</td> <td>s4884077</td> <td>Chr13</td> <td>77441896</td> <td>0.059</td> <td>0.313</td> <td>0.040</td> <td>9.94E-06</td> <td>0.812</td> <td>0.172</td> <td>0.420</td> <td>0.388</td> <td>0.081</td> <td>0.842</td> <td>0.166</td> <td>0.001</td>	Chr14 82206283 7.58E-06 0.320 1.20E-04 0.004 0.520 0.894 0.588 0.080 5.11E-05 0 and in NSCL/P in the European GWAS sample analysis 0.424 0.009 0.139 Failed QC in replication 0.11E-05 0 and in NSCL/P in the European GWAS sample analysis 0.424 0.003 0.041 0.467 0.648 0.402 0.604 0.026 0 Chr1 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.604 0.026 0 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.914 0.095 6 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.6155 0 Chr3 103247757 0.633 3.31E-06 0.007 0.0355 0.559 0.853 0.899 0.962 0 Chr5 76663192 <	s4884077	Chr13	77441896	0.059	0.313	0.040	9.94E-06	0.812	0.172	0.420	0.388	0.081	0.842	0.166	0.001
Chr20 61209037 9.05E-06 0.424 0.009 0.139 Failed QC in replication Failed QC in replication and in NSCL/P in the European GWAS sample analysis 0.046 0.648 0.402 0.604 0.576 0.233 Chr1 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.604 0.233 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.879 0.914 0.095 6.31E-05 0.111 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.095 6.31E-05 0.104 Chr5 4959824 0.349 1.62E-06 0.070 0.134 0.391 0.684 0.732 0.113 0.640 0.040 0.040 0.040 0.040 Chr5 76653162 0.271 787-06 0.070 0.113 0.655 0.040 0.040 0.040 0.040 0.040 <	Chr20 61209037 9.05E-06 0.424 0.009 0.139 Failed QC in replication and in NSCL/P in the European GWAS sample analysis 0.746 0.003 0.041 0.467 0.648 0.402 0.604 0.026 0 Chr1 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.604 0.026 0 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.860 0.199 0.615 0 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 0 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.853 0.962 0 Chr5 76663192 0.221 7.82E-06 0.007 0.035 0.382 0.795 0.795 0 0 0 0.962 0 0 0.662 0.962	s1777697	Chr14	82206283	7.58E-06	0.320	1.20E-04	0.004		0.894	0.588	0.080	5.11E-05	0.370	0.001	0.001
ant in NSCL/P in the European GWAS sample analysis Chr1 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.604 0.026 0.576 0.233 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.879 0.914 0.095 6.31E-05 0.111 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 0.006 0.085 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.853 0.899 0.962 0.004 0.040 Chr5 7663192 0.221 7.87F-06 0.070 0.134 0.301 0.684 0.732 0.113 0.855 0.009 0.092	ant in NSCL/P in the European GWAS sample analysis Chr1 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.004 0.026 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.879 0.914 0.095 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.899 0.962 Chr5 76663192 0.2211 7.82E-06 0.020 0.134 0.1391 0.653 0.962	s6011617	Chr20	61209037	9.05E-06	0.424	0.009	0.139		Failed QC in	replication			Failed QC ir	n replication	
Chrl 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.026 0.576 0.233 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.879 0.914 0.095 6.31E-05 0.111 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 0.006 0.085 Chr5 4959824 0.349 1.625 0.382 0.559 0.853 0.962 0.004 0.040 Chr5 76653192 0.271 7.87F-06 0.070 0.134 0.341 0.732 0.113 0.745 0.004 0.040	Chrl 161092955 7.86E-06 0.746 0.003 0.041 0.467 0.648 0.402 0.0604 0.026 Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.879 0.914 0.095 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.899 0.962 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.899 0.962 Chr5 76663192 0.221 7.82E-06 0.020 0.134 0.391 0.684 0.792 0.113 0.855	SNPs significant	in NSCL∕P in th€	e European GWAS sa	mple analysis											
Chr2 3154999 0.224 4.66E-06 0.035 0.243 0.166 0.879 0.914 0.095 6.31E-05 0.111 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 0.006 0.085 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.853 0.962 0.004 0.040 Chr5 76663192 0.271 7.87F-06 0.070 0.134 0.301 0.684 0.722 0.013 0.095 0.040	Chr2 3154999 0.224 4.66E-06 0.024 0.035 0.243 0.166 0.879 0.914 0.095 Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.899 0.962 Chr5 76663192 0.221 7.82E-06 0.020 0.134 0.391 0.653 0.962	s12132001	Chr1	161092955	7.86E-06	0.746	0.003	0.041	0.467	0.648	0.402	0.604	0.026	0.576	0.233	0.409
Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 0.006 0.085 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.853 0.899 0.962 0.004 0.040 Chr5 76663192 0.221 7.82E-06 0.020 0.134 0.391 0.684 0.732 0.113 0.855 0.009 0.092	Chr3 103247757 0.633 3.31E-06 0.001 0.869 0.795 1.000 0.860 0.199 0.615 Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.893 0.962 Chr5 76663192 0.221 7.82E-06 0.020 0.134 0.391 0.684 0.732 0.133 0.855	s10208086	Chr2	3154999	0.224	4.66E-06	0.024	0.035	0.243	0.166	0.879	0.914	0.095	6.31E-05	0.111	0.231
Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.853 0.899 0.962 0.004 0.040 Chr5 76663192 0.271 7.82E-06 0.020 0.134 0.391 0.684 0.732 0.113 0.855 0.009 0.092	Chr5 4959824 0.349 1.62E-06 0.007 0.035 0.382 0.559 0.853 0.969 0.962 Chr5 76663192 0.221 7.82E-06 0.020 0.134 0.391 0.684 0.732 0.113 0.855	s1513121	Chr3	103247757	0.633	3.31E-06	0.001	0.869	0.795	1.000	0.860	0.199	0.615	0.006	0.085	0.264
Chr.E 76663192 0.221 7.82E-06 0.020 0.134 0.391 0.684 0.732 0.113 0.855 0.009 0.092	Chr5 76663192 0.221 7.82E-06 0.020 0.134 0.391 0.684 0.732 0.113 0.855	s26883	Chr5	4959824	0.349	1.62E-06	0.007	0.035	0.382	0.559	0.853	0.899	0.962	0.004	0.040	0.132
		69687030	Chr5	76663192	0 221	7 82F-06	0000	0 134	0 391	0 684	0 732	0 113	0 255		0.002	0 0 0

Table 2 Associations results for parent-of-origin effects in oral clefts. showing the most significant SNP per locus in both discovery and replication cohorts

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				GWAS	GWAS data			Replication data	ion data			Combined data	ed data	
OI ANS	Chromosome	Position, hg18	P_{PAT}	P _{MAT}	P _{PofO}	P _{HET}	P_{PAT}	P _{MAT}	P _{PofO}	P _{HET}	PPAT	P _{MAT}	P _{PofO}	P _{HET}
rs12543318	Chr8	88937456	0.311	2.89E-06	0.006	0.103	0.548	0.010	0.143	0.336	0.269	7.85E-07	0.004	0.071
rs2948372	Chr8	12802775	0.146	0.016	0.006	6.13E-06	0.736	1.000	0.812	0.655	0.216	0.209	0.079	0.002
rs890519	Chr8	18949329	5.63E-06	0.776	0.003	0.085	0.494	0.119	0.554	0.267	4.18E-04	0.169	0.120	0.821
rs7922467	Chr10	129643702	0.013	0.025	0.001	7.10E-06	0.083	0.001	0.291	0.918	0.003	0.467	0.089	0.001
rs12426170	Chr12	119597971	0.001	0.365	0.003	8.60E-06	0.473	0.532	0.954	0.356	0.210	0.283	0.099	0.050
rs7988514	Chr13	19763521	0.221	2.89E-06	0.013	0.013	0.860	0.516	0.737	0.610	0.362	0.001	0.069	0.054
rs4429270	Chr15	89828778	0.944	5.82E-06	0.002	0.122	0.462	0.700	0.431	0.663	0.527	0.011	0.171	0.525
rs1610093	Chr19	4505774	0.051	0.028	0.004	8.81E-06	0.790	0.728	0.957	0.715	0.187	0.255	0.082	0.013
rs3852922	Chr20	53443077	0.139	6.67E-07	0.013	0.033	0.852	0.660	0.657	0.289	0.285	0.005	0.215	0.568
SNPs significan	t in NSCPO in the	SNPs significant in NSCPO in the combined European and Asian GWAS sample analysis	n and Asian GW	iene alnmes SV	lveis									
rs11678000	Chr2	229965612	9.43E-08	0.414	0.001	2.56E-04	0.858	0.869	0.808	0.739	7.74E-05	0.463	0.012	0.008
rs512778	Chr6	79401865	0.249	5.93E-06	0.014	0.250	0.056	0.317	0.543	1.000	0.045	1.24E-05	0.078	0.329
rs1483757	Chr12	116245923	3.82E-06	0.115	0.022	0.076	0.448	0.917	0.636	0.414	2.78E-05	0.179	0.034	0.058
rs6539608	Chr12	80976335	1.000	4.50E-06	0.001	0.039	0.768	0.069	0.294	0.639	0.864	1.56E-06	0.001	0.050
Abbreviations: GW clefting; PAT, pate In bold: SNPs tha	AS, genome-wide assi rnal; PofO, parent-of- t pass our discovery c.	Abbreviations: GWAS, genome-wide association studies; HET, heterozygous; LD, linkage clefting; PAT, patemal; PoIO, parent-of-origin; SNP, single-nucleotide polymorphism. In bold: SNPs that pass our discovery criteria of $P<1\times10^{-5}$ and show and $P\leq0.01$ in	neterozygous; LD, eotide polymorphis and show and $P \leq$	linkage disequilibri sm. 0.01 in the replica	lisequilibrium; MAT, materr the replication cohort.	disequilibrium; MAT, maternal; NSCLP, non-syndromic cleft lip with or without cleft palate; NSCPO, non-syndromic cleft palate only; NSOFO, non-syndromic orofacial in the replication cohort.	Indromic cleft	lip with or wit	hout cleft palà	ate; NSCPO, n	on-syndromic clef	t palate only; NSC	JFC, non-syndro	nic orofacial

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Table 2 (Continued)

influence of the two parental genomes on risk of OFCs in the child. Our stratified analyses by ethnic groups and type of OFC showed the number of loci showing a maternal-specific transmission bias equaled or exceeded the number of paternal-specific signals, with a significant excess of maternal-specific signals observed in three categories. Although this approach cannot discern the underlying mechanism, this observation suggests overall maternally inherited alleles exert a more significant effect on the risk of OFCs in offspring than do paternally derived alleles. In this regard, it is interesting to note effects of maternal genotype and maternalspecific environmental modifiers such as smoking, drinking and vitamin intake during pregnancy on oral cleft susceptibility have been previously reported in various studies.^{2,10-19} However, it should be noted that the parental asymmetry tests we applied here do not specifically test for maternal effects which would be expected to occur via alterations of the in utero environment. Calculation of genomic inflation factors for the MAT and PAT tests showed a small increase for the MAT compared with the PAT in all categories, this increased inflation factor in the MAT does not discern the underlying cause, being consistent with either a true excess of biological associations with the maternally inherited alleles, or alternatively differing population structure between the two parental populations.³⁵ In addition, comparison of the 64 SNPs used in replication analysis with two online catalogs of known imprinted loci (http://igc.otago.ac.nz/home.html and http://www.geneimprint.com/) showed that none are located within 1 Mb of any known imprinted loci. However, we do note that one study recently reported TBX6, the most significant locus identified in our genome-wide screen for PofO effects in OFCs, as showing evidence of imprinting in placental tissue.³⁶ As a result, we suggest that further studies of this locus in OFCs are warranted.

Although we utilized four different tests (MAT, PAT, PofO and HET), we did not apply any multiple testing correction for several reasons. First, as the PofO and HET tests share the same underlying data used by the MAT and PAT these four tests are not independent. Second, although we utilized a combined significance threshold of $P < 5 \times 10^{-8}$, our primary filter in the discovery cohort was to select SNPs showing $P_{PofO} < 0.05$, and thus the MAT and PAT tests were only applied to a relatively small fraction of the total genotypes available. Third, the use of a two-stage discovery and replication design in an additional cohort of \sim 1200 trios of European descent, should be considerably more robust than any previous studies of PofO effects in OFC.

Both the method we have used, and that used by Shi et al²⁰ are heavily dependent on SNPs that tag loci showing PofO-specific transmission biases. Using this assumption, we can identify some types of PofO effects, but not all. We also analyzed our discovery and replication data using a conventional TDT which does not consider parental origin. In this analysis, we observed three loci yielding nominal replication with both $P_{\text{TDT}} < 0.01$ in the discovery cohort and $P_{\text{TDT}} < 0.05$ in the replication cohort (combined discovery plus replication TDT *P*-values ranging from 2.4×10^{-4} to 3.3×10^{-7}). It should be noted that our MAT and PAT tests are essentially a modification of the TDT in which only one half of the genotypes inherited by each child are considered. When considered as individual tests, they are also capable of detecting risk alleles that do not exhibit PofO effects, although with reduced power compared with the conventional TDT. For example, as can be seen in Figure 2, previously identified OFC risk loci such as the 8q24 locus⁵ were detected, but as these show significant P-values in both the MAT and PAT tests strong PofO effects at these markers can be excluded. Therefore, a reasonable

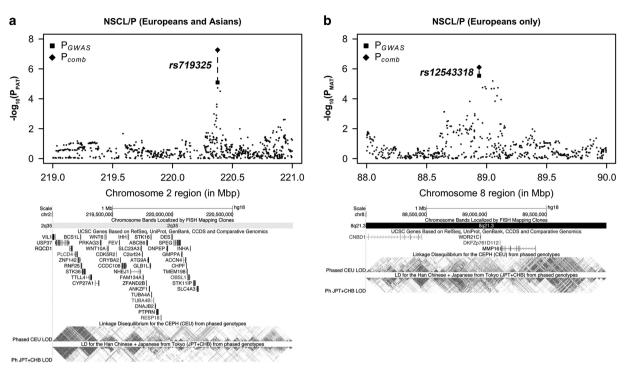


Figure 4 Loci on 2q35 and 8q21.3 show suggestive evidence of PofO effects in NSCL/P in both discovery and replication cohorts. (a) A paternal-specific signal at SNP rs719325 on chromosome 2q35 in combined European and Asian analysis (P_{GWAS} : $P_{\text{PAT}} = 8.07 \times 10^{-6}$, $P_{\text{MAT}} = 0.69$, P_{comb} : $P_{\text{PAT}} = 5.43 \times 10^{-8}$, $P_{\text{MAT}} = 0.12$). (b) A maternal-specific signal at SNP rs12543318 on chromosome 8q21.3 in European-only analysis (P_{GWAS} : $P_{\text{PAT}} = 0.31$, $P_{\text{MAT}} = 2.89 \times 10^{-6}$, P_{comb} : $P_{\text{PAT}} = 0.27$, $P_{\text{MAT}} = 7.85 \times 10^{-7}$). The squares and diamond represent *P*-values from the discovery GWAS and combined analysis, respectively.

alternative interpretation of our results is that some or all of the loci we detected with suggestive PofO effects might simply represent weak risk loci for OFC, and which manifest in our data in only one of the two parental tests by chance due to insufficient power to reliably detect these effects in both MAT and PAT tests. As such, we suggest some of the loci we identify represent interesting candidate regions for future studies of OFC.

In conclusion, our study provides suggestive evidence for PofO effects in susceptibility to OFC, identifying several loci showing a parental transmission bias, and an overall excess of maternal-specific association signals in the genome. Given abundant evidence supporting a role for non-Mendelian and transgenerational inheritance patterns in a variety of different diseases and conditions,⁹ we suggest similar analyses considering parental origin of risk alleles will likely reveal novel PofO effects contributing to many human phenotypes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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